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EFFECTS OF WATER SOLUBLE FRACTION OF CRUDE OIL AND DIETARY SUPPLEMENTATION OF ROSEMARY ON COMMON CARP (*CYPRINUS CARPIO*)

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ABSTRACT

This study was conducted to investigate the effects of water soluble fraction (WSF) of crude oil and dietary supplementation of rosemary on some physiological parameters of common carp (*Cyprinus carpio*). Common carp (mean weight = 30.41±2.28g) was exposed to (0, 3.57 and 7.14 ml/L) of WSF of crude oil and fed diets containing (0 and 0.1 %) of rosemary for 8 weeks. The experiment was arranged in completely randomized design (CRD) and data were analyzed through one-way ANOVA. The value of WG, SGR and survival were significantly ($P \leq 0.05$) decreased by exposure to WSF of crude and significantly ($P \leq 0.05$) enhanced by adding rosemary to exposed fish diet, while the FCR exhibited an opposite trend. The level of RBC, HGB, Hct, RBC, MCV, MCH, MCHC, WBC, LYM, GRA, MON, GOT and GPT were significantly ($P \leq 0.05$) decreased by exposing fish to WSF of crude oil, while the PLT, MPV cholesterol and LDL levels showed an opposite trends. Adding rosemary to exposed fish diet led a significant increase in the level of WBC, LYM, MON, MCH and MCHC. The results of this study showed that the WSF of crude oil have adversely affected the physiological parameters in common carp and dietary supplementation of rosemary could be one of the good approaches to reduce its negative effects.

Keywords: WSF of crude oil, *Cyprinus carpio*, growth performance, haematological parameters, biochemical parameters, rosemary

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INTRODUCTION

Crude oil spillage is a major environmental problem that occurs often throughout oil exploration and exploitation (Olajuyigbe *et al*., 2020). Iraq is blessed with abundant petroleum, and the daily exploitation and same lead to discharge of petroleum and its products into our aquatic ecosystem (Shlimon *et al* ., 2020). Crude oil, or petroleum, has an organic origin and is categorized as light, medium or heavy, depending on the dominant type of hydrocarbon, i.e., aromatic compounds comprising one benzene ring, polynuclear aromatic compounds comprising two or more benzene rings, or aliphatic-rich (long- or short-chain) compounds (Ibemenuga, 2013). Petroleum may also contain other compounds, such as sulfur, nitrogen, and oxygen, as well as trace metals, such as nickel and vanadium (Santos *et al*., 2016). Aquatic organisms are more likely to be exposed to water soluble fractions (WSFs) than other components of petroleum (Dighiesh *et al*., 2019).

The water soluble fraction (WSF) of crude oil is a complex and toxic mixture of hydrocarbons that aquatic organisms directly encounter in oil spills. WSF plays an important role in the toxicity of crude oil to aquatic organisms (Lari *et al*., 2016). The adverse effects of crude oil on growth performance and feed utilization in different species of fish have been reported (Omoregie and Ufodike, 1999; Omoregie and Ufodike, 2000; Udofia, 2010: Sharaf and Abdel-Tawwab, 2011; Abdel-Tawwab, 2012)) in tilapia, (Nwabueze and Agbogidi, 2010; Olaifa, 2012; Onwurah *et al.,*2013; George *et al*,. 2014; Fakolujo *et al*., 2018; Ajah and Ukutt, 2018: Olajuyigbe *et al*., 2020) in african catfish, (Lockhart *et al*.,1996) in rainbow trout and (Nasir and Hantoush, 2010; Anwar *et al*., 2020) in common carp *Cyprinus carpio*. 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: Anwar *et al*., 2020).

Haematological parameters such as Hb, Hct, RBC ,WBC 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 previous studies with different fish species (Omoregie, 1998; Awoyinka *et al*., 2011; Abdel-Tawwab,2012; Onwurah *et al*., 2013; Eriegha *et al*., 2017) in tilapia and (Ajah and Ukutt 2018;

Khoshbavar Rostami and Yelghi, 2020) in African catfish.

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). Eriegha *et al*. (2017) reported that exposed fish to a water soluble fraction of crude oil induced a variety of alterations on biochemical parameters in juveniles of nile tilapia *O. niloticus*. Furthermore, Khoshbavar Rostami and Yelghi (2020) found a significant reduction in ALP when giant sturgeon, *Huso huso* exposed to acute level of crude oil.

As the components of oil vary among different oil fields, oil from different regions have different toxicological effects, and different aquatic species have different sensitivities to a particular oil (Lari *et al.,* 2016). Therefore, extrapolating toxicology data from one oil field to another may not be reliable and a set of toxicology studies should be performed on the local fish species.

Medicinal plants and their bioactive compounds, including alkaloids, phenolic compounds, and steroids have been successfully applied in aquatic organisms to promote appetite, growth performance, stress responses and immunity (Abdel-Tawwab *et al*., 2018; Reyes-Cerpa *et al*., 2018; Elumalai *et al*., 2019). Herbs have been reported to promote various functions like growth (Shalaby *et al*., 2006), appetite stimulation, resistance to stress (Citarasu, 2010), immune responses (Erg¨un *et al*., 2011) in fish culture due to the many active components they contain. Rosemary (*Rosmarinus officinalis*) is a woody herb belonging to the Lamiaceae, and is native to the mediterranean region. Rosemary and its oil derivates have a long history in traditional medicine; however, limited information is available regarding applications in aquaculture (Hoseinifar *et al*., 2020).

Dietary supplementation could be one effective way of negating the negative impact of water soluble fraction of crude oil on farmed fish and improve the growth performance, feed utilization, fish hematological and serum biochemical parameters. For instance, dietary supplementation of rosemary extracts at tilapia (*Oreochromis mossambicus*) diets resulted in improved and enhanced some nonspecific immunity indicators in comparison to a test diet without rosemary inclusion (Yilmaz *et al*., 2011). In another study, rosemary extract (*Rosemaria officinalis*) was added to african catfish (*Clarias gariepinus)* test diets (Turan and Yiğitarslan, 2016). The results revealed that growth performance, feed utilization and body composition were better than no supplemented rosemary. Furthermore, Hassan *et al*. (2018) indicated that dietary turmeric (*Curcuma longa*), rosemary (*Rosmarinus officinalis*) and thyme (*Thymus vulgaris*) as feed additives improve the nutrient utilization, growth performance, carcass composition and some immuno-hematological parameters in Nile tilapia (*Oreochromis Niloticus*). More recently, Yousefi *et al*. (2019) have shown that dietary supplementation of rosemary leaf powder improved growth performance, enhance antioxidant and immunological parameters, and mitigate the negative effects of crowding stress on common carp fingerlings. However, to date, no study has been carried out to evaluate the effects of dietary supplementation of rosemary (whole plant) on growth performance and fish hematology, blood biochemistry in fish reared in water contaminated with crude oil.

The present study aimed to evaluate the effects of exposed common carp (*Cyprinus carpio*) of water soluble fraction of crude oil and dietary supplementation of rosemary leaf powder to assess growth performance, feed utilization and general physiology.

MATERIALS AND METHODS

The current work was conducted from 10th of August 2019 to 5th of October 2019. Research work was performed in the the aquaculture unit (close system), Grdarasha station, College of Agriculture Engineering Sciences, Salahaddin University- Erbil, Kurdistan Region -Iraq.

Experimental fish: Common carp (*Cyprinus carpio L.*) was sourced from Ankawa hatchery station, Erbil, Kurdistan Region, Iraq. Fish were transported to the aquaculture unit (close system), Grdarasha station, College of Agriculture Engineering Sciences, Salahaddin University- 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±2.28g). 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.

Experimental conditions: Rectangular polyethylene tank with dimensions (30 cm× 38 cm× 65 cm) (each with 0.074 m² capacity) was used for designing the current close system project for fish treatment. Fish exposed to sub-lethal levels (0, 3.57 and 7.14 ml/L) of WSF for 8 weeks. Each of these tanks was equipped with one inlet pipe (to form dissolved oxygen in tanks) all pipes connected with electric aerator.

Dissolved oxygen was measured daily by using the 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 of 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^{-2}) were determined in the Lab.

Water-soluble fraction (WSF) of the 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.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.

Diet formulation: 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% rosemary leaf powder supplementation for one of them. Both diets were formulated to be isonitrogenous (38%), isolipidic (8%) and isocaloric (19MJkg–^{l}).

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: A total of 96 juvenile common carp (mean weight= 30.41±2.28 g) divided into 12 rectangular polyethylene tanks (8 fish per tank, 2 tanks per group of fish). The experiment was arranged in Completely Ramdomized Design (CRD). There were six gruops of fish three gruops of fish exposed to 0% , 25% and 50% of sublethal (0, 3.57 ml\L and 7.14 ml\L) of WSF of crude oil fed control diet and other three gruops of fish exposed to 0, 3.57 ml\L and 7.14 ml\L WSF of crude oil fed diet supplemeted with 0.1% of rosemary leaf powder.

Haematological and Biochemical Analysis: At the end of the trial, fish were euthanised and blood collected from 6 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 collected 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 placed and centrifuged at 3,500 rpm for 15 minutes and the supernatant serum collected and put in labeled ineppendrof tubes stored at -80 ̊C until for biochemical tests. For haematological analysis WBC, LYM, MON, GRA, HGB, MCH, MCHC, RBC, MVC HCT, PLT and 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 (TG), 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 at105°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 at550°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).

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

Ingredient g kg-¹	C	R
Soybean	530	530
Corn	150	150
Fishmeal	100	100
Premix	25	25
Soya oilaaw	50	50
Wheat flour	100	100
Wheat bran	15	15
Vitamin Premix	11	11
Enzyme	1	1
Mineral premix	20	20
Rosemary	-	1
Proximate composition (%)		
Moisture (%)	7.79±0.42	8±0.28
Protein (%)	38.67±0.24	41.25±1.19
Lipid (%)	6.46±0.35	7±0.28
Ash (%)	6.4±0.21	6.66±0.31
NFE (%)	39.4±014	37.09±0.14

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
R= Rosemary
Nitrogen-free extracts (NFE %) = 100-(Ash+ moisture+ crud fat+ crude protein)
C= Control diet
R= Diet supplemented 0.1 % of rosemary

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/cm^3	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	19622.192

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

No	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

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/FL³ 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: Data on (please give details of parameters here) were analyzed through one-way ANOVA and diffences among treatments were evaluated using Duncan's Multiple Range Test (*P<0.05*). All results are expressed as mean values ± Standard error (±SE). Statistical analyses were conducted by SPSS statistics version 26 for windows (SPSS Inc., an IBM company, copyright 1989-2019**)**.

RESULTS AND DISCUSSION

Growth performance and body proximate composition: The results of the present study showed that weight gain (WG), specific growth rate (SGR) and survival rate were significantly *(P≤0.05)* decreased and feed conversion ratio (FCR) increased in exposed groups compared with unexposed groups (Table 4). Poor growth performances in the exposed groups could be attributed to the effects of the soluble fractions of crude oil, 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 groups indicated the inability of the exposed fish to convert feed consumed into required body protein (Sunmonu and Oloyede, 2006). The other possible reason for poor growth performance in the exposed fish may be due to immediate use up of protein content in feeds combat oxidative stress rather than being utilized for the new protein formation and increasing weight (Fakolujo *et al*., 2018). Furthermore, it is possibly reflective to reduce feed intake and thus lower body weight (Nwabueze and Agbogidi, 2010). Moreover, relatively the low survival rate through possible disease contagion observed in test fish exposed WSF concentration 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 knowledge on the effects of water soluble fraction of crude oil has on common carp is so far limited; however, there are reported studies on other farmed fish species. For example, the omnivorous tilapia fish (*Oreochromis niloticus*) was exposed to a water soluble fraction of crude oil for 10 weeks. The response after the toxicity trial demonstrated that the fish growth performance significantly reduced compared with unexposed fish (Omoregie and Ufodike, 2000). Similarly, growth performance, feed utilization, fish appetite and survival were significantly reduced in Nile tilapia *Oreochromis niloticus (L.)* exposed to CPF (commercial petroleum fuel). Moreover, Nwabueze and Agbogidi (2010) stated that the water-soluble fractions of crude oil significantly reduced the growth performance of catfish *Heterobranchus bidorsalis* fingerlings. Additionally, Abdel-Tawwab (2012) reported that growth performance and feed utilization significantly reduced in Nile tilapia *Oreochromis niloticus L*. following the acute exposure to kerosene, gasoline and diesel. Fakolujo *et al*., (2018) also found that sublethal levels of WSF of crude oil had adverse effects on the growth performance in *C. gariepinus* juveniles. Nevertheless, the finding of the current study disagrees with findings of Onwurah *et al.*(2013) who indicated that crude oil pollution could be beneficial to tilapia fish at certain sub lethal doses. On the other hand, weight gain (WG), specific growth rate (SGR) and survival rate were significantly (*P≤0.05*) higher in groups of fish received diet supplemented rosemary compared to fish groups fed control diet while, feed conversion ratio (FCR) showed an opposite trend (Table 5). It is possible that this may be due to promote appetite, stimulates the secretion of pancreatic enzymes, important factors in nutrient digestion and assimilation (Frankic *et al.*, 2009). Reported studies of rosemary use in aquafeeds is limited, but a study carried out on tilapia (*Oreochromis mossambicus*) using rosemary gave increased specific growth rate, weight gain, feed utilization and survival values compared to no inclusion (Yilmaz *et al*., 2011). Moreover, Turan and Yiğitarslan (2016) reported that supplementing rosemary extract (*Rosemaria officinalis*) diets for African catfish (*Clarias gariepinus*) can significantly improve growth performance and feed utilization. Similarly, Hassan *et al*. (2018) found that the supplement rosemary to plant based diets for Nile tilapia (*Oreochromis Niloticus*) improves growth performance and feed utilization. More recently, Yousefi *et al*. (2019) showed that dietary supplementation of rosemary leaf powder improved growth performance, enhance antioxidant and immunological parameters, and mitigate the negative effects of crowding stress on common carp fingerlings.

Based on the present findings the highest whole body lipid (3.84±0.006 %) was recorded in group (25% WSF) which was significantly (*P≤0.05*) higher than all experimental groups. Whole crude protein and ash contents were significantly (*P≤0.05*) reduced in exposed groups compared with unexposed groups fed the same diet. Nevertheless, moisture contents was significantly (*P≤0.05*) increased in exposed fish compared with unexposed fish. Crude lipid content was significantly (*P≤0.05*) increased in fish exposed to 25%, while in 50% of WSF showed an opposite trend. On the other hand, dietary supplementation of rosemary modulated the toxicity induced by both doses of WSF in fish groups but not significant. Increasing moisture content with exposing fish to WSF of crude oil in the current study is in agreement with Lockhart *et al*. (1996) who found the same trend in the rainbow trout exposed to WSF of crude oil. Reduction in the level of crude protein in the body of fish is a common effect of WSF of crude oil. It is assumed that protein is catabolized to generate more energy to combat against stress. Furthermore, reduction in the crude protein level appeared as the main effects on reduction in all growth parameters of *C. carpio*, because the synthesis of protein is required for the elaboration of tissue, which in turn affects FCR. The results of the present study coinciding with the results of other studies where fish exposed to different contaminants in water. Majumder and Kaviraj (2019) found a significant reduction in crude protein and ash contents in *Oreochromis niloticus* exposed to sub-lethal concentrations of the insecticide. Similarly, Amin and Indulkar (2017) found that exposing freshwater fish *Cyprinus carpio* to sublethal toxicity of a synthetic pesticide significantly reduced fish ash and crude protein contents. Decreasing lipid content with exposing fish to 50% WSF of crude oil in the current study inagreement with (Amin and Indulkar, 2017; Majumder and Kaviraj, 2019). In comparison, the supplementation of rosemary to exposed diets produced an physiologicaly increase in crude protein content but not significant. This is agreed with Hassan *et al*. (2018) who found the same trends in protein content in Nile tilapia (*Oreochromis Niloticus*) fed diets supplemented rosemary. While, moisture contents were physiologically decreased with supplementing rosemary to the diets of exposed and unexposed groups. Ash content was significantly increased by supplementing rosemary to a diet of fish exposed to 50% of WSF of crude oil. This is coinciding with Yousefi *et al*. (2019) who indicated that dietary supplementation of rosemary leaf powder did not significantly affect moisture, protein, lipid and ash contents in common carp fingerlings exposed to crowding stress. Further agreement with present study is Turan and Yiğitarslan (2016) who did not find any significant change in whole body protein and lipid content with dietary supplementation of rosemary extract (*Rosemaria officinalis*) to African catfish (*Clarias gariepinus*) diet.

Table 4: Growth performance and feed utilization of common carp (*Cyprinus carpio*) fed the experimental diets and exposed of WSF for 8 weeks. (n=2)

Parameters	Control	Control + R	25% WSF	25%WSF +R	50% WSF	50% WSF+R
WG (g)	50.57±1.43^{c}	57.12±1.12^{d}	43±0.75^{b}	46.59±0.99^{bc}	38.62±1.37^{a}	47.49±0.83^{c}
SGR %	1.36±0.04^{b}	1.54±0.008^{c}	1.2±0.008^{ab}	1.36±0.62^{b}	1.17±0.71^{a}	1.37±0.053^{b}
FCR	1.75±0.06^{ab}	1.57±0.009^{a}	1.94±0.032^{bc}	1.84±0.06^{b}	2.11±0.08^{c}	1.78±0.02^{b}
Survival %	100±0.00^{b}	100±0.00^{b}	62.5±12.5^{a}	75±12.5^{a}	62.5±12.5^{a}	75±12.5^{a}
K-Factor %	1.8±0.06^{a}	1.89±0.00^{a}	1.89±0.04^{a}	2.06±0.16^{a}	2±0.075^{a}	2±0.03^{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 common carp (*Cyprinus carpio*) fed the experimental diets and exposed of WSF (n=2).

Parameters	C	C +R	25% WSF	25%WSF +R	50% WSF	50% WSF+R
Moisture (%)	75.63±0.54^{ab}	75.39±1.18^{b}	77.54±0.04^{b}	76.26±0.03^{ab}	77.62±0.25^{b}	76.02±0.38^{ab}
Crude protein (%)*	12.94±0.28^{c}	13.13±0.62^{c}	10.6±0.01^{a}	12.04±0.01^{ab}	11.69±0.13^{b}	12.41±0.19^{ab}
Crude lipid (%) *	3.36±0.07^{bc}	3.38±0.16^{bc}	3.84±0.006^{d}	3.62±0.004^{cd}	3.07±0.03^{a}	3.32±0.02^{ab}
Ash (%) *	6.18±0.13^{b}	6.16±0.29^{b}	5.60±0.009^{a}	5.60±0.007^{a}	5.4±0.06^{a}	5.82±0.09^{ab}

Data are presented as mean ± S.E.
Data in the same row with different subscript are significantly different *(P≤0.05)*
*Dry matter basis.

Water quality: All the recorded dissolved oxygen concentrations, temperature and pH were adequate to support fish. A large number of studies have shown that WSF of crude oil can reduce water quality and thereby the overall well-being of aquatic organisms (Santos *et al*., 2016). The values of the water quality parameters indicated that WSF of crude oil adversely affected the water quality variables in the culture systems. pH level was not significantly (*P≥0.05*) affected with exposing to WSF of crude oil table 6. This is disagreement with the findings of Eriegha *et al*. (2019) who found a significant reduction in pH level after the introduction of water soluble fractions of crude oil. Awoyinka *et al*. (2011) also found a significant reduction in pH of the test vessel from 8.64 ± 0.03 to 5.50 ± 0.08 following the exposure of crude oil. Turbidity, temperature, salinity, total dissolved solid, chloride, potassium, calcium, nitrate levels increased significantly (*P≤0.05*) with exposing to both sub-lethal concentration of WSF of crude oil in comparison with control group table 6. These observations were similar to those of other workers (Obasohan *et al.,* 2011: Giari *et al*., 2012: Nwabueze and Agbogidi, 2010). Total alkalinity, magnesium, sodium and electro conductivity (EC) levels increased significantly (*P≤0.05*) with exposing to 50% sub-lethal concentration of WSF of crude oil, while this trend was not significant with 25%. However, dissolved oxygen, total hardness and sulfate levels reduced significantly (*P≤0.05*) with exposing to sub-lethal concentration of WSF of crude oil Table 6. Edema and Asagba (2007) reported similar reductions in DO after exposure to the different levels of WSF of crude oil. Awoyinka *et al*. (2011) also found a significant reduction in dissolved oxygen of the test vessel from 6.28 ± 0.07 to 3.01 ± 0.06 following the exposure of crude oil. 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. Sulfate levels also reduced significantly by exposing to sub-lethal concentrations of WSF of crude oil. This is in agreement with Eriegha *et al*. (2019) who found a significant reduction in level sulfate after the introduction of water soluble fractions of crude oil.On the other hand, dietary supplementation of rosemary modulate significantly the turbidity (16.18±0.18), sodium (96.16±0.52), potassium, total hardness, total alkalinity (173.33±2.33) and sulfate toxicity induced by WSF in fish groups Table 6.

Table 6. Mean values for water quality parameter (n=3).

Characteristics	Unit	C	C+ R	25%WSF	25%WSF+R	50% WSF	50% WSF+R
Turbidity	NTU	4.2± 0.17^{a}	3.9± 0.11^{a}	15.81± 0.26^{c}	15.25± 0.15^{b}	16.9± 0.15^{d}	16.18± 0.18^{c}
Temperature	˚C	24.35± 0.1^{a}	24.56± 0.19^{ab}	24.96± 0.08^{bc}	24.95± 0.13^{bc}	25.28± 0.21^{c}	25.25± 0.23^{c}
PH	-	7.77± 0.11^{a}	7.88± 0.04^{a}	7.8± 0.07^{a}	7.76± 0.13^{a}	7.55± 0.23^{a}	7.63± 0.13^{a}
Salinity	Ppt	0.19± 0.002^{a}	0.19± 0.005^{a}	0.21± 0.005^{bc}	0.20± 0.003^{b}	0.22± 0.003^{c}	0.22± 0.005^{bc}
DO	mg\L	7.53± 0.12^{d}	7.5± 0.11^{d}	6.46± 0.14^{bc}	6.56± 0.13^{c}	6.06± 0.04^{a}	6.16± 0.1^{ab}
Resistivity	Ohm	2.5± 0.018^{ab}	2.53± 0.008^{b}	2.49± 0.008^{a}	2.48± 0.005^{a}	2.49± 0.003^{a}	2.48± 0.008^{a}
TDS	Ppm	265.6± 2.12^{a}	265.6± 2.08^{a}	271.83± 0.68^{bc}	270.83± 0.04^{b}	274.86± 0.33^{bc}	275.35± 0.63^{c}
EC	$\mu S.cm^{-1}$	0.253± 0.001^{a}	0.252± 0.001^{a}	0.253± 0.001^{a}	0.254± 0.0006^{a}	0.260± 0.001^{b}	0.261± 0.002^{b}
Cl^{-}	mg\L	20.96± 0.6^{a}	20.6± 0.35^{a}	23.05± 0.42^{b}	22.9± 0.4^{b}	23.6± 0.26^{b}	24.01± 0.12^{b}
Na^{+}	mg\L	96± 0.11^{a}	97.66± 0.24^{b}	96.43± 0.26^{ab}	95.91± 0.15^{a}	103.66± 0.88^{c}	96.16± 0.52^{a}
K^{+}	mg\L	5.2± 0.05^{b}	3.8± 0.05^{a}	5.6± 0.028^{c}	6.3± 0.25^{d}	8.11± 0.1^{e}	8.83± 0.06^{f}
Ca^{+2}	mg\L	20± 0.1^{ab}	19.5± 0.3^{b}	20.11± 0.1^{abc}	21.06± 0.31^{c}	20.93± 0.41^{bc}	20.86± 0.42^{bc}
Mg^{+2}	mg\L	33.13± 0.19^{a}	33.06± 0.18^{a}	33.3± 0.27^{a}	33.45± 0.18^{a}	34.66± 0.35^{b}	35.01± 0.54^{b}
TH	$mg.CaO_3.l^{-1}$	198.33 ±2.02^{c}	198± 1.15^{c}	183± 1.52^{a}	194.33± 1.7^{bc}	187± 1.15^{a}	191.66± 0.88^{b}
T. Alkalinity	$mg.CaCO_3.l^{-1}$	160± 1.15^{a}	156.66 ±0.88^{a}	157± 0.57^{a}	157.66± 0.33^{a}	168± 1.52^{b}	173.33± 2.33^{c}
$NO^{-}{}_{3}$	mg\L	20± 0.29^{a}	19± 0.15^{a}	23.01± 0.52^{bc}	22.31± 0.50^{b}	24.31± 0.5^{c}	23.81± 0.48^{c}
SO_4^{-2}	mg\L	129± 0.57^{d}	122.66 ±0.33^{ab}	122± 0.57^{ab}	123.66± 0.88^{b}	120.66± 0.88^{a}	125.66± 0.33^{c}

Data are presented as mean ± S.E.
Data in the same row with different subscript are significantly different (*P≤0.05*)

Blood parameters: Blood is an excellent indicator of toxic stress and analysis of hematological parameters in fish are widely used to assess the toxic stress and functional status of the animal health (Kavitha *et al*., 2010). The WBC, absolute LYM, LYM%, absolute MON, MON%, GRA, Hb, RBC, MCH, MCHC, MCV and Hct value of fish exposed to sublethal concentration of WSF of crude oil were significantly (*P≤0.05*) decreased Table 7. However, PLT and MPV value of *C. carpio* exposed to sublethal concentration of WSF of crude oil and fed control diet were significantly (*P≤0.05*) increased Table 7. Since RBC and Hb are directly involved with oxygen circulation, reduction in both might result in oxidative stress, which in turn affects growth performance in exposed fish, albeit negatively. Significant reduction in RBC, Hb and Hct observed in test fish exposed to WSF of crude oil could be precursory to severe anaemia and eventual death. 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 (Saravanan *et al*., 2011). Further lysing of erythrocytes due to toxicant stress may also lead to a reduction in hemoglobin and haematocrit values in the fish (Kori-Siakpere and Ubogu, 2008). The high percentage of immature red blood cells in the circulation might be the reason for MCV and MCH decrease in the present investigation. The observed low concentration of MCHC during acute treatment might have resulted from a decrease in Hb synthesis due to the toxic action of lindane or swelling of erythrocytes by which blood O_2 transport capacity is increased when fish were subjected to less effective gas exchange (Saravanan *et al*., 2011). The general reduction of the blood parameters indicates anaemic condition caused by exposure of *Cyprinus carpio* to toxicant. According to Rostem and Soltani (2016) crude oil can affect RBC, causing a hemolysis by a disruptive effect on the erythropoietic tissues of spleen and kidney. The deleterious alteration of haematological parameters of fish reported in this study which is due to stress induced by environmental pollutant (water soluble fraction of crude oil) have been reported by several researchers with different fish species such as (Omoregie,1998; Abdel-Tawwab ,2012; Onwurah *et al*., 2013;Eriegha *et al*,. 2017) in Nile tilapia *Oreochromis niloticus* L (Awoyinka *et al.,* 2011; Ajah and Ukutt 2018; Fakolujo *et al.,* 2018) in african catfish *Clarias gariepinus*, (Rostami and Yelghi, 2018) in giant sturgeon, *Huso huso,* (Awoyinka *et al*., 2011) in mudfish *Clarias anguillaris* and (Hantoush, 2010) in common carp *Cyprinus carpio.* On the other hand, some blood data disagreement with Sharaf and Abdel-Tawwab (2011) who found a significant increase of RBC, Hb in Nile tilapia, *Oreochromis niloticus (L.)* following exposure to commercial petroleum fuels (CPF). Conroversly, Lari *et al*. (2016) did not find any significant difference in hemoglobin and hematocrit in Caspian roach, Rutilus caspicus after water soluble fraction of crude oil. Khoshbavar Rostami and Soltani (2016) reported that MCV, MCH and PCV significantly increased in juvenile Persian sturgeon, *Acipenser persicusn* following the acute exposure of crude oil.

The findings of current study indicated that the rosemary is a positive dietary additive to improve hematological parameters and reduce the adverse effects of exposed fish to a water soluble fraction of crude oil. The WBC, absolute LYM, LYM%, absolute MON, MON%, MCH and MCHC value were significantly (*P≤0.05*) increased with supplementing rosemary to a diet of exposed fish. A significant (*P≤0.05*) reduction was found in PLT and MPV value of *C. carpio* exposed to sublethal concentration of WSF of crude oil and fed diet supplanted rosemary Table 7. Although, the effects of supplementation of rosemary to common carp exposed of WSF of crude oil on hematology have not been investigated up to date. The findings of the current study can be compared with few studies which have been conducted with different toxicants and different species of fish. The current findings is coinciding with Hassan *et al.* (2018) who found a significant increase in hematocrit and WBC with dietary supplementation of rosemary to the diet of Nile Tilapia (*Oreochromis Niloticus*). Gültepe *et al.* (2014) reported that dietary supplementation of rosemary to tilapia (*Oreochromis mossambicus*) diet increased some haematological indicators (WBC, RBC, PCV, neutrophils and monocyte).

Glutamic oxaloacetic transaminase (GOT) glutamic-pyruvic transaminase (GPT) serum components can be generally used to assess the tissue damage of the liver and kidney (Agrahari *et al*., 2007). Fish exposed to sub-lethal concentrations of crude oil showed a significant increase (*P≤0.05*) in the level of GOT and GPT compared with the control groups table 8. This finding is coinciding with some studies. Vedel *et al*. (1998) reported a considerable increase in the GOT and GPT of rainbow trout, *O. mykiss*, exposed to ammonia. De Smet Blust (2001) also indicated that GOT and GPT activities increased in *C. carpio* exposed to cadmium. Similarly Shin *et al*. (2016) indicated that GOT and GPT significantly increased in rockfish, Sebastes schlegelii, during thermal stress by the ammonia exposure. Han *et al*. (2019) found significant increase in GOT and GPT in juvenile starry flounder, *Platichthys stellatus*, following exposure to varying arsenic concentrations. Alkaline phosphate (ALP) is the biomarker enzymes for liver functions (Raibeemol and Chitra, 2018). Alkaline phosphate was significantly decreased with exposing fish to 50% of sublethal concentrations of WSF of crude oil in the current study . This is in agreementwith Jahanbakhshi and Hedayati (2013) finding who reported that alkaline phosphatase significantly decreased in juveniles beluga exposed to water-soluble fraction (WSF) of crude oil. Khoshbavar Rostami and Soltani (2016) also indicated that ALP enzyme decreased significantly in juvenile Persian sturgeon, *Acipenser persicus* acute exposed to crude oil. Additionally, Inyang *et al*. (2018) reported that ALP significantly decreased in *Clarias gariepinus* exposed to toluene (produced water component). Khoshbavar Rostami and Yelghi (2020) found a significant reduction in ALP when giant sturgeon, *Huso huso* exposed to cute level of crude oil. However, disagree with Eriegha *et al*. (2017) who found a significant increase in ALP when juvenile *Oreochromis niloticus* exposed to four sub-lethal concentrations (30, 45, 60 and 75% of water soluble fractions of crude oil. 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 this study, cholesterol was significantly (*P≤0.05*) decreased in fish exposed to both doses of WSF of crude oil table 8.

The results in the current study is agree with (Ajah and Ukutt 2018) study who found significant reduction in cholesterol and triglyceride when clariid catfish juveniles exposed to water soluble fraction of crude oil. Triglyceride usually is used to identify animal health (Mohiseni *et al*., 2016). The level of triglycerides was significantly (*P≤0.05*) increased in fish exposed to 50% of WSF of crude oil and fed diet supplemented rosemary compared with other groups of fish Table 8. This is disagreement with (Ajah and Ukutt 2018) who found a significant reduction in triglyceride when clariid catfish juveniles of water soluble fraction of crude oil. Bagheri *et al*. (2018) also indicated that level of triglyceride significantly decreased in juveniles Asian Sea bass (*Lates calcarifer*) exposed to crude oil. Our finding in triglyceride are agree with Jahanbakhshi and Hedayati

(2013) who indicated that triglyceride significantly increased in juveniles beluga exposed to water-soluble fraction (WSF) of crude oil.The level of LDL was significantly (*P≤0.05*) decreased in exposed fish table 8. No significant (*P≥0.05*) difference was found in value of HDL with exposing fish to of WSF of crude oil Table 8.

The value HDL, GOT, GPT and alkaline phosphatedid were not significantly (*P≥0.05*) changed with supplementing rosemary to exposed fish. However, cholesterol and LDL value were significantly (*P≤0.05*) increased with supplementing rosemary to diet of fish exposed to both doses of WSF of crude oil. Triglycerides was significantly (*P≤0.05*) increased with supplementing rosemary to diet of fish exposed to 50% of sublethal concentration of WSF of crude oil table 8. This is coinciding with Kumar *et al*. (2010) who reported that triglycerides and ALP significantly increased in common carp (*Cyprinus carpio L.*) fingerlings fed with differently detoxified Jatropha curcas kernel meal. Similarly, Kim *et al*. (2013) found a significant reduction in the cholesterol and triglycerides with supplementation of dietary Spirulina and Quercetin to juvenile olive flounder *Paralichthys olivaceus*. Uncumusaoğlu (2018) also found that the level TRG and ALP significantly decreased in common carp (*Cyprinus carpio*) fed different levels of zeolite. Aditionally, Imanpoor *et al*. (2011) showed that concentration of cholesterol decreased in those fish which were exposed to levels of chloramine in common carp.

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

Parameters	Control	Control + R	25% WSF	25%WSF +R	50% WSF	50% WSF+R
WBC (×10^9\/L)	97.45±0.35^{d}	97.65±0.34^{d}	71.22±1.93^{b}	76.22±1.11^{c}	60.59±2.03^{a}	69.07±1.28^{b}
LYM# (×10^9/L)	70.05±1.47^{c}	70.43±1.06^{c}	41.35±1.51^{a}	51.47±1.02^{b}	39.3±1.38^{a}	49.78±1.4^{b}
MON# (×10^9/L)	7.53±0.16^{c}	7.71±0.19^{c}	6.67±0.17^{b}	7.76±0.10^{c}	5.05±0.26^{a}	7.18±0.18^{bc}
GRA# (×10^9/L)	17.54±0.56^{b}	18.18±0.26^{b}	14.77±0.43^{a}	14.68±0.29^{a}	13.82±0.42^{a}	14.7±0.42^{a}
LYM%	73.97±0.55^{c}	75.4±0.76^{c}	67.95±1.16^{b}	70.07±0.76^{b}	64.47±1.14^{a}	68.57± 0.87^{b}
MON%	8.26±0.08^{c}	8.35±0.12^{c}	7.35±0.15^{a}	8.12±0.06^{bc}	7.7±0.31^{ab}	8.2±0.14^{bc}
GRA%	22.25±0.6^{b}	22.54±0.86^{b}	14.67±0.32^{a}	14.82±0.18^{a}	13.95±0.29^{a}	14.87±0.28^{a}
HGB g\dL	10.4±0.07^{b}	10.51±0.17^{b}	9.28±0.16^{a}	9.57±0.23^{a}	9.14±0.17^{a}	9.51±0.06^{a}
MCH pg	19.19±0.34^{b}	19.25±0.40^{b}	14.92±0.62^{a}	18.65±0.45^{b}	13.72±0.31^{a}	14.57±0.31^{a}
MCHC g\dL	93.9±2.36^{b}	93.82±2.19^{b}	68.57±1.04^{a}	88.97±10.92^{b}	62.72±2.05^{a}	84.7±2.58^{b}
RBC (×10^{12}/L)	5.25±0.21^{b}	5.3±0.29^{b}	2.98±0.11^{a}	3.35±0.22^{a}	3.07±0.16^{a}	3.15±0.12^{a}
MCV fL	230±1.82^{b}	233.75±1.65^{b}	214.25±4.9^{a}	211.25±3.03^{a}	208.25±0.47^{a}	209.75±4.02^{a}
HCT %	23±0.18^{b}	23.37±0.16^{b}	21.42±0.49^{a}	21.12±0.3^{a}	20.82±0.04^{a}	20.97±0.4^{a}
PLT (×10^9/L)	4528± 282.02^{a}	4538.25± 161.9^{a}	7320.25± 449.1^{b}	4433.5± 137.27^{a}	9648± 177.89^{c}	6545.5± 274.8^{b}
MPV fL	8.1±0.31^{a}	8.13±0.23^{a}	8.92±0.41^{ab}	8.95±0.78^{ab}	10.16±0.27^{c}	8.28±0.32^{a}

Data are presented as mean ± S.E.

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 common carp (*Cyprinus carpio*) blood samples offed the experimental diets and exposed of the WSF (n=3).

Parameters	C	C+R	25% WSF	25%WSF+R	50% WSF	50%WSF+R
Cholesterol mg/dl	122.33±2.84^{c}	115.33±2.6^{bc}	82.33±3.17^{a}	94.66±11.6^{a}	99.33±1.76^{ab}	120.33±2.4^{c}
GOT U/L	161±1.15^{a}	155.33±3.92^{a}	291.33±19.7^{b}	278±6.65^{b}	397.66±17.14^{c}	391.33±7.12^{c}
GPT U\L	6±0.57^{a}	5.66±1.2^{a}	11.66±2.9^{b}	8±1.73^{ab}	9±0.57^{ab}	10.46±0.4^{ab}
TG mg\dL	179±19.85^{a}	162.33±7.8^{a}	158.33±10.58^{a}	148.66±7.05^{a}	176±14.57^{a}	284.66±4.91^{b}
ALP U\L	23.33±5.23^{ab}	16.33±2.4^{a}	23.33±1.2^{ab}	32.33±3.92^{b}	19±1.00^{a}	23.03±0.92^{ab}
HDL mg\dl	19.66±2.66^{a}	21±1.52^{a}	20±2.3^{a}	22.66±1.76^{a}	18.66±1.2^{a}	20.93±0.66^{a}
LDL mg\dl	67±1.52^{f}	59.33±2.4^{e}	23.33±0.88^{b}	29.33±0.88^{c}	18±1.15^{a}	53.8±1.8^{d}

Data are presented as mean ± S.E.

Data in the same row with different subscript are significantly different (*P≤0.05*)

Conclusion: It can be concluded that water-soluble fractions of crude oil have a highly significant effect of reducing the growth performance, feed utilization, blood heamatology, blood chemistry, carcass composition of common carp. The study has also shown dietary supplementation with rosemary is more effective at ameliorating these adverse effects of WSF of crude oil and enhancing fish immunity. Interestingly, herbs especially rosemary are potential treatments for reduction of the harmful toxic effects of water soluble fraction on aquaculture fish.

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