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Influence of Feeding Fish the Dried-Treated Sewage on Physiological Responses and Histological Structure of the Liver

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ABSTRACT

Fish are one of the most extensively distributed organisms in the aquatic environment. A field study was conducted for 102 days at feeding period. A 100 fish/net-Hapa (Nile tilapia, silver carp, common carp and African catfish) were stocked at a rate of 1: 1: 1: 1 using a polyculture system. Two experimental net-Hapa/treatment were used, one treatment as a control fish fed a commercial diet and the second one fish were fed a Dried Sewage Sludge (DSS), product of treating sanitary and agricultural drainage of Al-Reiad city, Kafr El-Sheikh, Egypt). At the end of the experiment, blood samples were withdrawn for measuring the hematological and serum biochemical parameters, also liver samples were taken from each fish species for histopathological examinations. From the obtained results, it was clear that DSS feeding did not negatively affect the blood picture in general, although the significantly elevated values of transaminases activity and triglycerides concentration. The DSS feeding led to some drastic histological alterations in the liver structure. Conclusively, the presence of some pollutants from agricultural and urban drainages whether in the rearing water or in DSS that can negatively affect fish health, production and quality as well as could be inter the food chain and threat human health; so, it is recommended to give more concern on food and water quality (environmental friendly) used in aquaculture to offer more safe products for human consumption.

Key words: Fish, sewage sludge, blood parameters, histopathology, polyculture

INTRODUCTION

Different wastes are frequently used in fish feeding or as organic fertilizers for fish ponds (Agouz and Gomha, 2011) from plant, animal and/or variable sources (Abdelhamid and Soliman, 2013). Sewage is a black coloured foul smelling fluid or semifluid containing organic and inorganic solids in dissolved and suspended forms carried away from residences, business houses and industrial establishments for final disposal (Ghosh *et al*., 1985). Recycling of nutrients in sewage is a potential intervention for food and nutrition scarcity and poverty reduction, especially in the developing countries (Dale, 1979). In order to recycle sewage, traditional practices (agriculture, horticulture and aquaculture) have been undertaken by several countries (Edwards, 1996). The sewage-fed fish cultures are important (Jana, 1998). An emphasis has been given for the recovery of nutrients from wastewater. It is an urgent call of the day that proposed action would be aquaculture by standardization of the treatment of domestic sewage. Sewage can be applied to farms in raw, partially treated or fully treated states. Furthermore, each of the types can be diluted with raw water. Dilution and treatment improve the quality of sewage (Pandey and Srivastava, 2009).

Sewage may be converted into income and high quality protein through fish culture (Meadows, 1983). Sewage reuse is a preferred method of sewage disposal to minimize treatment costs and obtain maximum agricultural and fish culture benefits from the residual nutrients in the sewage (WHO., 1989). Fish production in sewage-fed ponds is a common practice around the world. The use of sewage for fish culture has the potential to defray the costs of sanitation and sewage treatment processes (Ghosh, 1983). The quality of fish grown in sewage fed ponds remains as one of the major concerns, though the practice followed by farmers for nearly a century demonstrates the robustness of the system. Some studies conducted to understand the microbiological and chemical qualities of fish grown in sewage fed ponds indicate that the fish are safe to consume (Nandeesha, 2002).

Availability of inexpensive and readily available feed for fish is a major constraint for the development of aquaculture especially in developing countries. In view of this, it is important to formulate fish diet using readily available and cheap materials. Moreover, wide spectrum of feeding habits of fish permits the use of different materials, which are cheap or nearly valueless (Saini and Sharma, 1999). Municipal sewage sludges have been advocated by several investigators, as a suitable dietary supplement in aquaculture because of the protein contained in it (Pereira *et al*., 1996). But, the sewage sludge contains a variety of pollutants, such as biodegradable organic matter, heavy metals and pathogens and the arbitrary discharge of the sludge would bring heavy pollution to the environment (Lasheen and Ammar, 2009). Partially, the heavy metals in sludge have drawn more and more attentions because it can be accumulated along the food chains and create potential risks to animals and humans (Garcia-Delgado *et al*., 2007). Hence, other researchers discourage their usage for a variety of reasons, the most common being the presence of heavy metals and pesticides that accumulate in various organs, thereby hampering growth in fish (Yadav *et al*., 2002).

Fish are used as excellent indicator of aquatic pollution due to their high sensitivity to environmental contaminants which may damage certain physiological and biochemical processes when contact with the organs of fishes (Saravanan *et al*., 2011). Fish exposure to chemical contaminants induces lesions in different target organs, especially in liver (Miranda *et al*., 2008). According to Figueiredo-Fernandes *et al*. (2006), liver plays an important role in vital functions in basic metabolism and it is the major organ of accumulation, biotransformation, excretion and detoxification mechanisms of contaminants in fish. Thus, many authors have investigated the impacts of sewage on fish abundance and/or condition (Otway, 1995; Svanberg, 1996) but few have conducted field experiments. The field experiments have generally placed freshwater fish, such as salmonids and cyprinids, in cages adjacent to sewage outfalls and these fish generally suffered high mortalities (Kakuta and Murachi, 1997). Additionally, some farmers in the experimental region (Kafr El-Sheikh governorate, Egypt) or in nearly other regions developed a simple treatments of using domestic sewage or other wastes for fish culture, to meet the growing demand for fish in this thickly populated Egyptian city, as well as to convert wastes into consumable products. Thus, a field study was conducted to investigate the effects of feeding the basal diet (control) and Dried Sewage Sludge (DSS) on hematological, blood biochemical parameters and histological structure of the liver of four fish species (Nile tilapia, common carp, silver carp and African catfish), reared in the covered-net Hapas for 102 days.

MATERIALS AND METHODS

Experimental management: This study was conducted during the summer season in a private fish farm at Tolompat 7, Al-Reiad city, Kafr El-Sheikh governorate, Egypt. Nile tilapia, silver carp and African catfish were purchased from a private fish farm, Kafr El-Sheikh governorate, Egypt. While, common carp were purchased from Integrated Fish Farm at Al-Manzala city (General Authority for Fish Resources Development, Ministry of Agriculture), Al-Dakahlia governorate, Egypt. Fish were stocked into a covered-net Hapa for two weeks as an adaptation period and fed a basal (commercial) diet during this period.

Fish were distributed into two experimental treatments (two net Hapas for each); in the first treatment fish were fed the basal diet (as a control group), whereas in the second treatment, fish were fed DSS. Each net Hapa (8×3×1 m) were constructed and implanted in an earthen pond (irrigated from agricultural drainage). Four fish species with an average initial body weight of Nile tilapia (T), *Oreochromis niloticus* 178.0±3.5 g, common carp (Cc), *Cyprinus carpio* 232.0±2.7 g, silver carp (Sc), *Hypophthalmichthys molitrix* 344.0±4.3 g and African catfish (Cf), *Clarias gariepinus* 408.0±3.2 g were distributed in the experimental covered-net Hapas. A total of 100 fish/net-Hapa were stocked at a rate of 1: 1: 1: 1 for each fish species. Throughout the experimental period, water quality parameters in each net-Hapa were measured weekly, including temperature (via a thermometer), pH-value (using Jenway Ltd., Model 350-pH-meter, Staffordshire ST15 0SA, UK) and dissolved oxygen (using Jenway Ltd., Model 970-dissolved oxygen meter, Staffordshire ST15 0SA, UK). Average values of water temperature were 25.0±3.0°C, pH 8.80±0.7 and dissolved oxygen 2.66±0.3 mg L^{-1}.

The basal diet was purchased from El-Morshedy Brothers manufacture for animal, poultry and fish feeds, Meet Ghamr city, Al-Dakahlia governorate, Egypt. It is contained yellow corn, soybean meal (44%), wheat bran, fish meal (65%), corn gluten (60%), lime stone, common salt, dicalcium phosphate and molasses and had not less than 25% crude protein, 3% crude lipids, 3935 kcal gross energy kg^{-1} diet and not more than 5.30% crude fiber, according to the manufacture's formula.

The treated DSS was obtained from the duple stage treatment project (Sanitary Drainage Station Al-Reiad city, Kafr El-Sheikh governorate, Egypt). The tested diet and DSS were offered once daily (10:00 am) at 5% of the total fish biomass at each Hapa. The feed quantity was adjusted each 21 days according to the actual fish body weight changes. The proximate chemical analysis for the basal diet (control) and DSS was carried out according to AOAC (2004) and illustrated in Table 1. The DSS seems to be CP-richer than the control diet; yet DSS contains very high percentage of ash and very low percentages of EE and total carbohydrate percentages comparing to the control diet.

Blood samples: At the end of the experiment, five fish/species/net-Hapa were randomly taken and anaesthetized. Fishes were transferred in a small plastic tank containing 10 L water supplemented with 3 mL pure clove oil (dissolved in 10 mL absolute ethanol) as a natural anesthetic material. For

Table 1: Chemical composition of the tested commercial diet and dried sewage sludge

	Dry matter basis (%)	
Composition	Control (C) diet	DSS
DM	92.75	92.12
CP	25.75	30.21
EE	3.56	0.89
Ash	6.42	41.54
Total carbohydrate	64.27	27.36

DM: Dry matter, CP: Crude protein, EE: Ether extract, DSS: Dried sewage sludge the hematological parameters analysis, blood samples, (5 mL of whole blood at each collection), were collected from the fish by puncturing caudal venous with a syringe needle. Blood samples were kept in small plastic vials containing heparin-anticoagulant. Other blood samples were collected in dried plastic tubes and centrifuged for 20 min at 3500 rpm to obtain the blood serum. Serum samples were kept in deep freezer (-20°C) until the biochemical analysis was carried out.

Blood analyses: Heparinized whole blood were used for the determination of hematological parameters (hemoglobin, Hb; Red Blood Cells (RBCs), Packed Cell Volume (PCV) Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cells (WBCs) and blood platelets (PLT) using Swelab® Alfa Auto counter, USA. While, serum biochemical parameters were determined using commercial kits (Diagnostic System Laboratories, INC, USA), according to the standard methods of urea (Fawcette and Scott, 1960), creatinine (Newman and Price, 1999), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Reitman and Frankel, 1957). Total protein and albumin were determined according to the methods described by Tietz (1990) and Doumas *et al.* (1971), respectively. The concentration of serum globulin was obtained by subtracting the albumin from the serum total protein concentration (Doumas and Biggs, 1972). Total cholesterol was assesses following the method described by Trinder (1969), triglycerides according to McGowan *et al.* (1983), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) according to NCEP (1995).

Histological examination: At the end of the experiment, samples of livers were taken from three fish/species/net Hapa, trimmed and fixed (preserved) in 10% phosphate buffered formalin (saline solution) for 4 days. Washed with tap water for 24 h and then dehydrated in ascending grade of ethyl alcohol, cleared in xylol, embedded for 2 h in 2 changes of paraffin wax (melting point 56°C) and sectioned at 5 μ thickness, stained with enriches acid haematoxylin and eosin stains (H and E), mounted in Canada balsam, then examined microscopically (Roberts, 2001).

Statistical analysis: All numerical data was statistically analyzed by SAS (2001) software package for users guide (version 9.2), with factorial design (2×4) using the following model:

$$Yijk = \mu+Li+Mj+LMij+eijk$$

where, Yijk is the data of hematological and serum biochemical measurements, of fish species treated with dietary DSS or the control diet, μ is the overall mean, Li is the fixed effect of the two dietary treatments (control diet and DSS), Mj is the fixed effect of four fish species; Nile tilapia (T), common carp (Cc), silver carp (Sc) and African catfish (Cf), LMij is the interaction effect between the dietary treatments and fish species and eijk is the random error. All ratios and percentages were arcsine-transformed prior to statistical analyses. The differences between mean of treatments were compared using Tukey's post hoc significant test and differences were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Hematological parameters: Regardless of fish species, only RBCs, WBC and PLT reflected significant differences between both dietary treatments (Table 2) with higher values for DSS fed fish. However, Cf gave significantly higher values for most of the hematological parameters

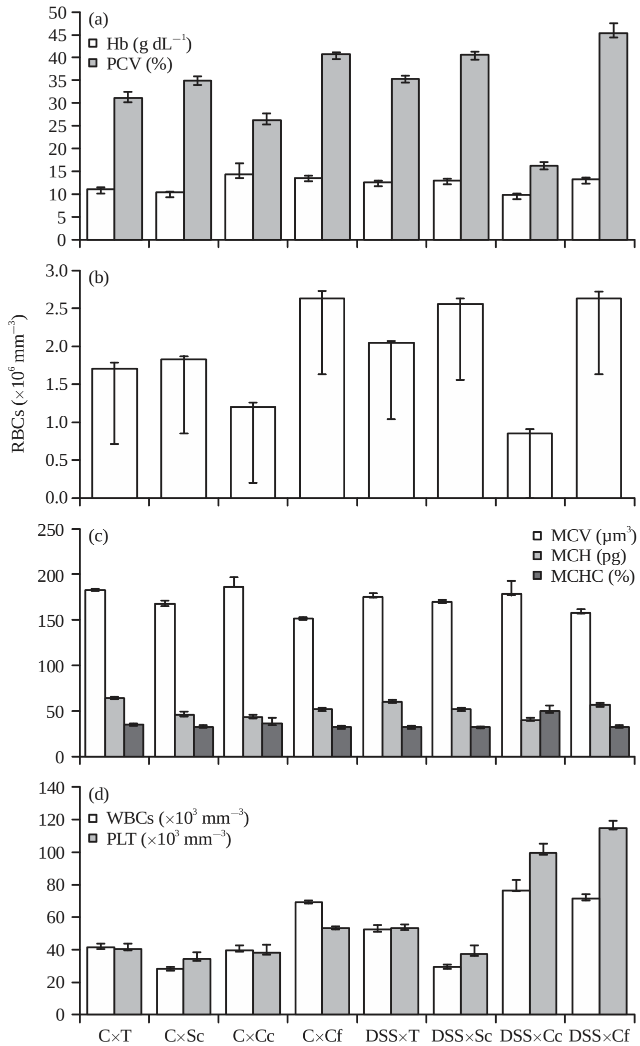

Fig. 1(a-d): Effect of interaction between dietary treatments and fish species on some hematological parameters, (a) Hb and PCV, (b) RBCs, (c) MCV, MCH and MCHC and (d) WBCs and PLT

referring to best tolerance among the studied fish species (Table 2). Except MCV, all other hematological parameters reflected significant interaction effects (Fig. 1a-d), where the highest Hb (Fig. 1a) and MCV (Fig. 1c) values were of Cf fed the control diet but DSS fed Cf gave the highest MCHC (Fig. 1c) and WBCs (Fig. 1d), Cf fed DSS had also the highest RBCs (Fig. 1b) and PCV (Fig. 1a) and only T fish fed the control diet had the highest MCH value (Fig. 1c).

Hematological changes were detected in fish exposed to pollutants in laboratory studies. The present findings revealed that DSS feeding did not negatively affect the hematological parameters. Inversely with our results, the thrombocyte fraction significantly reduced in dabs (*Limanda limanda*) exposed to sewage sludge (Secombes *et al*., 1991, 1992). Also, goldfish (*Carassius auratus*) exposed to 50% raw and 100% treated sewages on day 30, showed lower values of RBCs, granulocytes and lymphocytes counts, activity of phagocytic cells, Hb and plasma protein concentrations than those of the control group. Similar changes in blood parameters were observed in gold fish exposed to 5% raw and 10% treated sewages for 30 days (Kakuta, 1997).

Table 2: Effect of dietary treatments and fish species on some hematological parameters

	Blood indices							
Treatments	Hb (g dL^{-1})	RBCs×10^6 (mm^{-3})	PCV (%)	MCV (μm^3)	MCH (pg)	MCHC (%)	WBCs ×10^3 (mm^{-3})	PLT×10^3 (mm^{-3})
C	12.39±0.68	1.84±0.13[b]	33.35±1.45	171.84±4.32	50.96±2.26	34.22±1.35	45.06[b]±3.95	41.95±2.34[b]
DSS	12.19±0.37	2.01±0.18[a]	34.46±2.91	170.22±3.81	51.87±2.05	36.57±2.39	57.64[a]±5.08	76.91±8.46[a]
p-value	0.726	0.0005	0.174	0.722	0.484	0.261	0.0001	0.0001
Fish species								
T	11.76±0.34[b]	1.87±0.07[c]	33.35±1.04[c]	179.0±2.03[ab]	61.93±1.07[a]	33.63±0.86[b]	47.33±2.35[c]	47.66±2.87[c]
Sc	11.66±0.52[b]	2.18±0.14[b]	37.86±1.15[b]	168.2±1.97[b]	48.60±1.99[c]	32.22±0.53[b]	28.85±0.83[d]	36.20±3.04[d]
Cc	12.16±1.35[ab]	1.03±0.07[d]	21.29±2.03[d]	182.4±7.92[a]	41.50±1.40[d]	43.20±4.46[a]	58.70±7.55[b]	69.41±12.12[b]
Cf	13.57±0.12[a]	2.62±0.05[a]	43.12±1.32[a]	154.5±2.00[c]	53.65±1.17[b]	32.53±0.58[b]	70.52±1.46[a]	84.45±11.82[a]
p-value	0.005	0.0001	0.0001	0.0008	0.0001	0.002	0.0001	0.0001

Mean in the same column for each category having different superscript letters are significantly different ($p \leq 0.05$), C: Control diet, T: Tilapia, Sc: Silver carp, Cc: Common carp, Cf: Catfish, DSS: Dried sewage sludge, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelets

Table 3: Effect of dietary treatments and fish species on kidney and liver functions parameters

Treatments	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})	AST (IU L^{-1})	ALT (IU L^{-1})
C	13.48±1.33	0.51±0.03[b]	50.02±4.96[b]	49.43±4.87[b]
DSS	12.83±0.71	0.66±0.05[a]	98.72±14.46[a]	102.20±16.04[a]
p-value	0.203	0.003	0.0001	0.0001
Fish species				
T	11.00±0.33[bc]	0.60±0.05[ab]	62.52±8.87[b]	62.39±6.21[b]
Sc	9.76±0.46[c]	0.60±0.06[ab]	42.23±4.66[c]	44.60±6.77[b]
Cc	20.36±1.18[a]	0.65±0.09[a]	141.9±21.17[a]	141.70±26.76[a]
Cf	11.50±0.27[b]	0.49±0.04[b]	50.82±1.63[c]	54.58±5.88[b]
p-value	0.0001	0.007	0.0001	0.0001

Mean in the same column for each category having different superscripted letters are significantly different ($p \leq 0.05$), C: Control diet, T: Tilapia, Sc: Silver carp, Cc: Common carp, Cf: Catfish, DSS: Dried sewage sludge, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Serum biochemical measurements: Regardless of fish species, serum creatinine concentration, AST and ALT activity were significantly increased with feeding fish DSS (Table 3) comparing with those fed the control diet. Regardless of dietary treatments, Cc gave significantly the highest values of urea, creatinine, AST and ALT. Whereas, Sc gave significantly the lowest values for urea, AST and ALT (Table 3). That means that both of Cc and Sc were more influenced with feeding DSS (which affected both liver and kidney functions) than T and Cf. The interaction (dietary treatment×fish species) effect on urea (Fig. 2a), creatinine (Fig. 2b), AST and ALT (Fig. 2c) was significant ($p \leq 0.0001$) and except urea, all other parameter's levels (creatinine, AST and ALT) were significantly at highest for Cc fed DSS; yet, control Cc gave the significantly highest urea concentration.

The measurement of suitable biomarkers in liver becomes useful and can give an idea about the health state of fish. The transaminases, AST and ALT are two key enzymes considered as a sensitive measure to evaluate hepatocellular damage (Ibrahim and Mahmoud, 2005). In different fish species including *C. gariepinus*, AST and ALT enzymes activity were found to increase in response to heavy metals (Mekkawy *et al*., 2011). An increase in plasma AST and ALT activities due to metals (Zn, Cu and Cd) was also found in experimental conditions (Zikic *et al*., 2001), as well as in fish chronically exposed to metals (Levesque *et al*., 2002). In addition, the present findings coincide with the reported histopathological lesions which revealed a marked degeneration and necrosis of hepatocytes as the elevation in transaminases activities may be attributed to liver injury (Aly *et al*., 2003). Moreover, Metwalli (2013) gave higher levels of creatinine and AST but lower values of urea and ALT for *O. niloticus* than given in the present study. In a study by Yang *et al*. (1993), *O. mossambicus* fed sludge (30%) supplemented diet did not impose any effect on both ALT and AST activities up to 30 days. However, at the end of the experiment the liver AST activity was

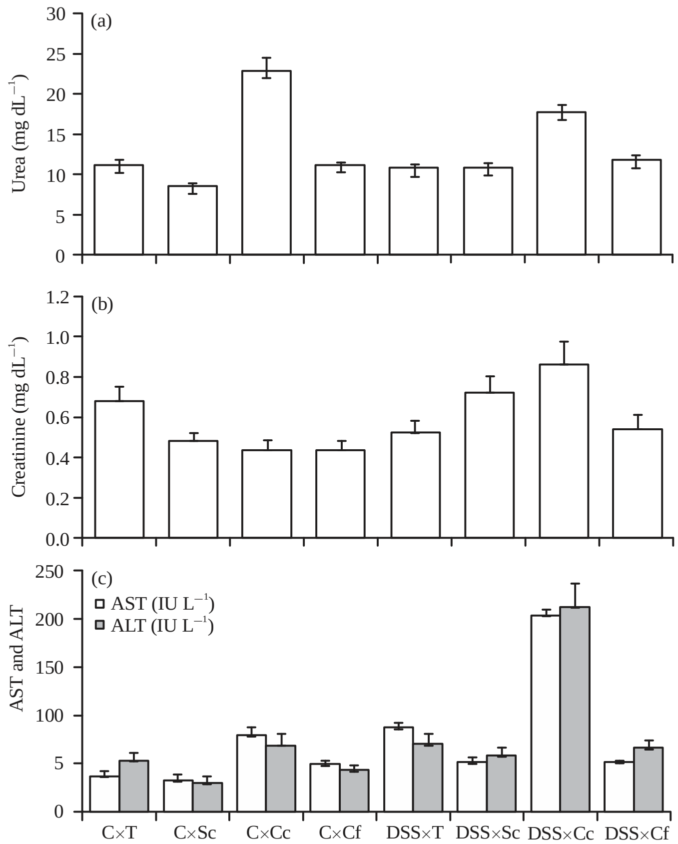

Fig. 2(a-c): Effect of interaction between dietary treatments and fish species on kidney and liver functions parameters, (a) Urea, (b) Creatinine and (c) AST and ALT

significantly increased ($p<0.05$) in fish receiving 10 and 30% of sludge supplemented diets. Inversely with our results, ALT activity was significantly decreased ($p<0.05$) in fish receiving 30% of sludge supplemented diet only, when compared to the control group.

Fish feeding DSS caused significantly higher globulin but lower albumin and albumin/globulin ratio values. Regardless of dietary treatment, T serum contained the highest total protein, globulin and albumin (Table 4). The interaction effect confirmed that the significantly highest total protein and globulin concentrations were realized with T fish fed DSS but the significantly highest albumin and albumin/globulin ratio values were found in T fish fed the control diet (Fig. 3).

Serum albumin and globulin have been used as indicators of healthy status of fish and considered as important indicators for the effect of pollutants in fish (Tayel *et al*., 2007). Exposure to sewage sludge also resulted in a decrease of serum total proteins (Secombes *et al*., 1992). Also, Houlihan *et al*. (1994) concluded that long-term exposure of common dab (*L. limanda*) to sewage sludge may have reduced protein growth, possibly through greater protein degradation in the tissues and increased protein degradation seems to have depressed immune responsiveness which agrees with the present findings on the experimental fish species exposed to DSS. A decrease in total proteins is always due to a low albumin level or to edema. Low serum albumin may be due to decreased formation in the liver because of severe liver damage. Also, serum protein have been used to understand the general state of health and biological mechanism of metabolism under pollutant stress (Saravanan *et al*., 2011). During stress conditions fish need more energy to detoxify

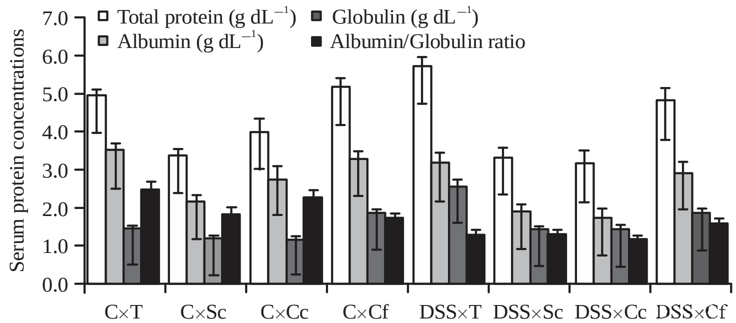

Fig. 3: Effect of the interaction between dietary treatments and fish species on serum protein concentrations

Table 4: Effect of dietary treatments and fish species on serum protein concentrations

Treatments	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})	Albumin/Globulin ratio
C	4.36±0.20	2.93±0.15^{a}	1.43±0.07^{b}	2.08±0.10^{a}
DSS	4.25±0.27	2.43±0.18^{b}	1.83±0.12^{a}	1.33±0.06^{b}
p-value	0.550	0.002	0.0001	0.0001
Fish species				
T	5.33±0.17^{a}	3.33±0.16^{a}	2.00±0.20^{a}	1.86±0.23
Sc	3.35±0.13^{b}	2.03±0.12^{b}	1.32±0.06^{b}	1.57±0.12
Cc	3.57±0.26^{b}	2.25±0.25^{b}	1.32±0.07^{b}	1.73±0.21
Cf	4.98±0.19^{a}	3.11±0.16^{a}	1.87±0.05^{a}	1.66±0.07
p-value	0.0001	0.0001	0.0001	0.205

Mean in the same column for each category having different superscript letters are significantly different ($p \leq 0.05$), C: Control diet, T: Tilapia, Sc: Silver carp, Cc: Common crap, Cf: Catfish, DSS: Dried sewage sludge

the toxicant and to overcome stress. So, due to these proteins in liver degrade and the serum protein level increase. Singh and Sharma (1998) reported decline in protein constituent in liver and increase in serum in different fish under stress of pollutants. Similarly, in the present study values of serum protein, albumin and globulin were increased of the experimental fish species (Table 4). This increase of these parameters goes in parallel with the elevation in the levels of water parameters studied and heavy metals concentrations as a result of pollution stress (Tayel *et al*., 2007).

Data of serum total cholesterol, triglyceride, HDL and LDL are given in Table 5. Regardless of fish species, feeding fish DSS resulted in significantly higher values of triglyceride, HDL and LDL comparing with those fed the control diet. Regardless of the dietary treatment, T fish represented the significantly highest concentration of both triglyceride and HDL but Cf contained the significantly highest levels of total cholesterol, HDL and LDL (Table 5). The significant interaction effect (Fig. 4) revealed that the highest total cholesterol and LDL concentrations were found with control Cf but T and Cf fed DSS gave the highest triglyceride and HDL levels, respectively.

The biochemical analyses and hematological parameters of fish rapidly respond to environmental conditions (Hardig and Hoglund, 1984). In the present study, the relatively high significantly levels of serum triglyceride, HDL and LDL of DSS group, compared to the control group (Table 5), could be related with the rise in the energy demands and the serum transport of fatty acids through HDLs. So, the tendency of cholesterol levels to rise as a function of sewage concentration could be related with to the possible increase in energy demand. The albumin levels in the vertebrates has been associated with fatty acid, divalent metallic cations transport and blood colloidosmotic pressure (Metcalf *et al*., 1998). The lipid transport in the fish is similar to mammals,

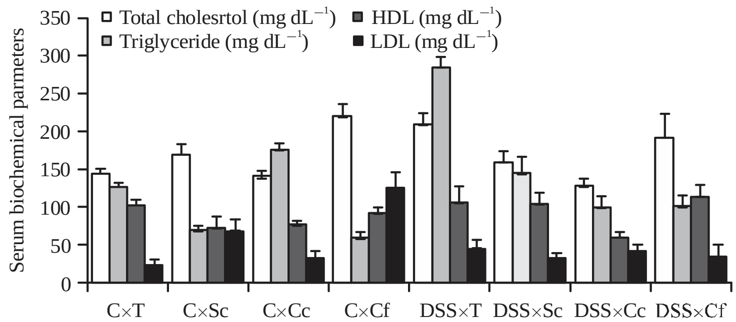

Fig. 4: Effect of the interaction between dietary treatments and fish species on concentrations of some serum biochemical parameters

Table 5: Effect of dietary treatments and fish species on concentrations of some serum biochemical parameters

Treatments	Total cholesterol (mg dL^{-1})	Triglyceride (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})
C	168.9±9.00	108.10±11.24^{b}	86.05±5.11^{b}	62.10±11.24^{b}
DSS	172.4±11.40	158.00±19.15^{a}	96.10±8.56^{a}	38.95±4.99^{a}
p-value	0.756	0.0001	0.009	0.002
Fish species				
T	177.0±13.16^{ab}	206.40±27.69^{a}	104.10±10.77^{a}	34.30±7.09^{b}
Sc	165.2±9.06^{bc}	107.80±16.33^{c}	88.10±11.17^{ab}	50.70±9.48^{b}
Cc	134.0±6.60^{c}	138.30±14.95^{b}	68.20±4.52^{b}	36.90±6.20^{b}
Cf	206.6±17.10^{a}	79.70±10.68^{d}	103.90±8.60^{a}	80.20±19.29^{a}
p-value	0.0008	0.0001	0.003	0.002

Mean in the same column for each category having different superscript letters are significantly different ($p \le 0.05$), C: Control diet, T: Tilapia, Sc: Silver carp, Cc: Common carp Cf: Catfish, DSS: Dried sewage sludge, HDL: High density lipoprotein, LDL: Low density lipoprotein

the Very Low Density Lipoprotein (VLDL) being the main transporter of triglyceride, whereas LDL and HDL are rich in cholesterol. In fish, the low levels or absence of albumin is compensated by fatty acid transport through HDL (Nanton *et al*., 2006). As in the present findings of serum triglyceride, HDL and LDL, similarly Kandemir *et al*. (2010) reported that when high mortality rate was observed for all of the experimental fish species (*Cyprinus carpio*, *Leuciscus cephalus*, *Capoeta trutta* and *Capoeta capoeta umbla*), a statistically significant increase was recorded for serum triglyceride. Inversely with the present results, triglyceride of *O. mossambicus* fed 10 and 30% of sludge supplemented diets was not significantly different (p>0.05) from fish fed the control diet (Yang *et al*., 1993).

Histopathological examination: The present histological findings of *O. niloticus* fed the control diet showed that normal hepatocytes and enlargement, slight congestion and infiltration of the blood vessel (Bv) (Fig. 5a). However, the histopathological examination of liver of *O. niloticus* fed DSS showing necrotic hepatocytes and enlargement, severe congestion and infiltration of the Portal Blood Vessel (PBV) (Fig. 5b). Figure 5c illustrated that *H. molitrix* fed the control diet showed normal hepatic lobules with normal hepatocytes and diffusion of melanomacrophage centers (MMCs). While, *H. molitrix* fed DSS showed large areas of necrotic hepatocytes, infiltration of BV, areas of degenerated hepatocytes (Fig. 5d).

Microscopically, *C. carpio* fed the control diet showed normal hepatic lobules with normal hepatocyte arrangement around the Central Vein (CV) adjacent with blood sinusoids (Fig. 5e). Meanwhile, *C. carpio* fed dried sewage sludge showed severe necrotic hepatocytes and severe

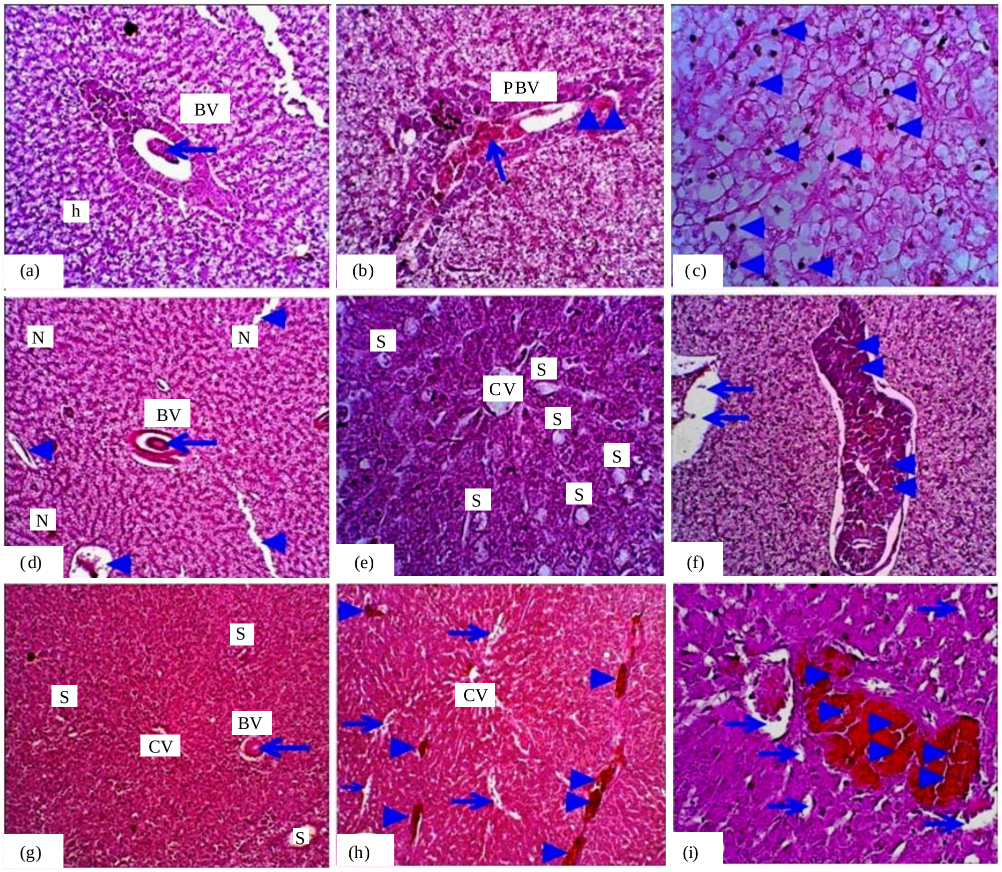

Fig. 5(a-i): Cross sections in liver of, (a) *Oreochromis niloticus* fed the control diet showing normal hepatocytes (h) and enlargement, slight congestion and infiltration (arrow) of Bv, (b) *Oreochromis niloticus* fed DSS showing necrotic hepatocytes and enlargement, severe congestion (arrows heads) and infiltration (arrow) of PBV, (c) *Hypophthalmichthys molitrix* fed the control diet showing normal hepatic lobules with normal hepatocytes and diffusion of MMCs (arrows heads), (d) *Hypophthalmichthys molitrix* fed DSS showing large areas of Necrotic (N) hepatocytes, infiltration (arrow) of BV and areas of degenerated hepatocytes (arrows heads), (e) *Cyprinus carpio* fed the control diet showing normal hepatic lobules with normal hepatocyte (h) arrangement around CV adjacent with blood Sinusoids (S), (f) *Cyprinus carpio* fed DSS showing severe Necrotic (N) hepatocytes and severe congestion, enlargement of PBV with monocytes, fibroblast infiltration (arrows heads) and large area of degenerated hepatocytes (arrows), (g) *Clarias gariepinus* fed the control diet showing normal hepatic lobules with normal hepatocyte arrangement around CV, adjacent with blood Sinusoids (S) and slight congestion of BV (arrow), (h) *Clarias gariepinus* fed DSS showing large areas of degenerated hepatocytes (arrows) around CV and diffusion of hemosiderin in the hepatic lobule (arrows heads) and (i) High magnification of (Fig. 5h) showing large areas of degenerated hepatocytes (arrows) and diffusion of hemosiderin in the hepatic lobule (arrows heads); a, b, d, e, f, g, h (×100, H and E stains), c and i (×400, H and E stains), Bv: Blood vessel, PBV: Portal blood vessel, MMCs: Melanomacrophage centers, CV: Central vein congestion, enlargement of PBV with monocytes, fibroblast infiltration and large area of degenerated hepatocytes (Fig. 5f).

Histological investigation of the liver of *C. gariepinus* fed the control diet showed normal hepatic lobules with normal hepatocyte arrangement around CV, adjacent with blood sinusoids, slight congestion of BV (Fig. 5g). However, *C. gariepinus* fed DSS showed large areas of degenerated hepatocytes around CV and diffusion of hemosiderin in the hepatic lobule (Fig. 5h). In addition, highly magnification (Fig. 5i) for the same treatment of catfish showed that large areas of degenerated hepatocytes and diffusion of hemosiderin in the hepatic lobule.

The liver histology is used as biomarker for the environmental pollution (El-Serafy *et al*., 2009). Several histopathological changes in the liver have been reported of fish exposed to a wide range of organic compounds, heavy metals, sewage, agricultural and industrial pollutants (Au, 2004; Abdel-Moneim *et al*., 2012). Liver lesions, such as perivascular and pericholangiar reactions as described here, are also reported in fish which were exposed to organic contaminants (Johnson *et al*., 1993), pesticides (Couch, 1975), or heavy metals (Sastry and Gupta, 1978). The mechanism could be a reactivity of toxic metabolites with the bile duct epithelium (Hinton and Lauren, 1990). So, it is not surprised in the present harmful effects of all fish species fed the control diet; concerning the hematological, blood biochemical parameters or histological structure of the liver may be related to fish rearing water quality in the experimental site. Where, the agriculture drained water was polluted with different types of pesticides, fertilizers and some heavy metal's residues not only in the experimental fish farm but also in all fish farms and fish hatcheries in Egypt according to the dishonorable Egyptian law No. 124/1983. Currently, this law prohibits aquaculture projects from drawing surface water, leaving more than 90% of the country's fish farms and fish hatcheries to operate on polluted agricultural drainage water. In addition, Vos *et al*. (2000) and Kolpin *et al*. (2002) reported that sewage effluents contain complex mixtures of chemicals such as natural and synthetic hormones, alkyl phenols, phthalates, bisphenol A, pharmaceuticals and some pesticides. Hence, these drastic effects of all experimental fish species fed DSS in the present study, may be due to the organic pollutants (PCBs, PBDEs and HBCDs) contamination in sludge, sediments and fish (Ilyas *et al*., 2013), as well as may be due to the long exposed time to DSS (102 days) in the present study. Hence, Moore *et al*. (1996) reported that exposure to sewage sludge has caused liver damage in fish.

The liver not only represents a central organ concerning basic metabolism (Gingerich, 1982) but is also a major site of the accumulation, biotransformation and excretion of toxic compounds (Meyers and Hendricks, 1985). Subsequently, hepatocytes respond to changes in the external and internal environments by alterations in both cellular structure and function (Wheater *et al*., 1985). Since, Ayas *et al*. (2007) described that necrosis lesions in liver of fish is one of the most important histopathological responses due to exposure to contaminants. Additionally, the principle of hepatic necrosis results from the presence of chemicals within cells causing disturbs on biochemical process as enzyme inhibition, failure on protein synthesis, carbohydrate metabolism, reactive oxidative species production, damages in cell membrane and failure of ATP synthesis (Rabitto *et al*., 2005).

Several histopathological changes have been reported in the liver of experimental fish in response to agricultural, sewage and industrial pollutants (Mohamed, 2003). The present study suggests a strong link between dietary DSS and histopathological lesions zin the liver. Similar results have been reported in *O. mossambicus* exposed to cadmium and zinc (Van Dyk *et al*., 2007), in *C. gariepinus* exposed to lead (Aly *et al*., 2003; Olojo *et al*., 2005); cadmium (Pantung *et al*., 2008), in freshwater catfish, *C. punctatus* exposed to 3 and 5 mg cadmium chloride for 15 and 45 days (Amin *et al*., 2013). Additionally, Mohamed (2008) reported that several histopathological alterations, including vacuolar degeneration with focal areas of necrosis in liver were observed in the studied tissues of fish as a result of the accumulated metals. Hence, those findings lend support to the observations of the present study. Generally, in the present study the slightly drastic effects on liver tissues in all fish species fed the control diet were detected. Where, these histopathological alterations in the liver of all fish fed DSS were markedly impaired. These severe alterations in the hepatocytes were confirmed by potential negative effects on hematological parameters regarding to the dietary treatments and fish species (Table 2), their interaction (Fig. 1) or blood biochemical parameters regarding to the dietary treatments, fish species (Table 3-5) or their interaction (Fig. 2-4) in both fish fed the control diet or treated by DSS.

CONCLUSION

The obtained results in the present study revealed that the feeding DSS led to slightly impaired effects on hematological parameters but it had significantly drastic effects on blood biochemical parameters compared to the control group. Also, the harmful histological alterations in the liver of all fish species fed DSS compared to the control group were detected. Consequently, no doubt these drastic effects related with the presence of some pollutants from agricultural and urban drainages whether in the rearing water or in the feeding DSS that can negatively affect fish health, production and quality, as well as could be inter the food chain and threat human health. Thus, it is recommended to give more concern on food and water quality (environmental friendly) used in aquaculture to offer safe products for human consumption.

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