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RESEARCH ARTICLE

EFFECTS OF CHRONIC EXPOSURE OF CADMIUM ON GROWTH PERFORMANCE, BIOACCUMULATION AND HEMATOLOGICAL PARAMETERS OF GENETICALLY IMPROVED FARMED TILAPIA

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ABSTRACT

This study was conducted to evaluate the chronic exposure of cadmium on growth performance, bioaccumulation, and hematological parameters of genetically improved farmed tilapia (GIFT). Two concentrations of cadmium (50 μgL^{-1} and 100 μgL^{-1}) with control were used. Twelve fish with an initial mean weight of 12.84 ± 0.53 g were randomly assigned quadruplicated treatments (total 144 fish) and the experiment lasted for six weeks. In the end, growth performance and feed utilization efficiencies were assessed by using % specific growth rate (%SGR), % average daily gain (%ADG) and feed conversion ratio. The pattern of Cd accumulation in different tissues and hematological parameters were examined. Mean body weight, %ADG, %SGR, hematocrit and packed cell volume were not affected by the treatments. A significantly higher survival rate (100 %) was observed in control followed by CD50 (87.50 %) and CD100 (78.13 %). The rate of Cd accumulation in various tissues was in the order of liver > gills > muscle > skin in each treatment. Among tested concentrations, the highest accumulation was observed in CD100 for each tissue. Both red blood and white blood cell count ($2.21 \pm 0.07 \times 10^6$ mm^{-3} and $468.00 \pm 6.61 \times 10^3$ mm^{-3} respectively) were significantly higher in control and it was lowest in CD 100 ($1.28 \pm 0.04 \times 10^6$ mm^{-3} and $205.19 \pm 8.94 \times 10^3$ mm^{-3}). The results of this study supported the conclusion that there is a significant effect of the chronic exposure to cadmium on the bioaccumulation and hematological parameters of the GIFT tilapia even at the ecologically relevant concentration.

Keywords: Cadmium exposure, Growth, Bioaccumulation, Hematological parameters, GIFT

INTRODUCTION

Sri Lanka consists of many man-made reservoirs, which are either perennial or seasonal. Most of the reservoirs are scattered in the dry zone of the country and are being used in the agriculture and aquaculture sectors. Run-off from the surrounding catchment area is the main source of water for these reservoirs. Most of the surrounding landscapes of these systems are agricultural fields, where intensive agricultural practices are being conducted. Therefore, the runoff to the reservoirs carries nutrients, chemical residues, pesticide residues and sediments, and acts as sinks for those inputs. Among those chemicals, heavy metals have been identified as one of the potential toxicants which cause a significant impact on the entire aquatic ecosystem, through bioaccumulation and bioconcentration. (Levit and Bozeman 2010). Compared to terrestrial organisms, biotic elements in aquatic systems are highly vulnerable to heavy metal bioaccumulation (Kumar and Singh 2010). This is a major environmental problem associated with the run-off in many seasonal reservoirs.

There is an array of heavy metals that cause significant impacts on the aquatic ecosystem, and cadmium has been identified as one of the most toxic forms of heavy metal among others (Borgmann *et al*. 2005). However, cadmium does not exist as its elemental form in nature,

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and thus, it is available as compounds such as cadmium oxide, cadmium chloride, cadmium sulphide, cadmium cyanide, cadmium carbonate and cadmium nitrate. Even at low concentrations, Cd causes toxic effects on all life, including plants, fish, birds, mammals and microorganisms (Eisler 1985). The application of phosphate fertilizers is one of the main sources of Cd for agricultural lands. Therefore, overuse of phosphate fertilizers in agricultural fields induces Cd accumulation in soil and consequently leaches to the downstream water bodies. Therefore, an elevated level of cadmium in water and soil increases its uptake by live organisms (Perera *et al.* 2015). As there is the potential bioaccumulation of Cd along the aquatic food chain, fish are highly vulnerable to associated toxic effects. However, the reported level of Cd in fish collected from Sri Lankan reservoirs ranged from 0.006 - 0.06 $mg.kg^{-1}$; wet weight (Bandara *et al*. 2008). Both waterborne and dietary exposure to Cd can cause toxic effects on fish.

At low concentrations, cadmium does not directly cause a toxic effect on the organisms. According to Ayoola *et al*. (2014), fish is in close contact with their environment, and are very susceptible to physical and chemical changes which are detected using hematological, physiological, and biochemical biomarkers. As waterborne exposure directly contacts the gills of fish, toxicological changes may be visible in their histology. Further, since fish are intimately associated with the aqueous environment, blood will reveal measurable physiological changes in the fish more rapidly than other biomarkers (Kefas *et al.* 2015). Even though the bioaccumulation and availability of Cd in Sri Lankan waters have been previously reported, information on the toxicological impact of Cd exposure on fish in the Sri Lankan context is not well documented. Therefore, the present study was designed to study the impacts of chronic exposure to Cd on growth performance, bioaccumulation, and hematological parameters of genetically improved farmed tilapia.

MATERIALS AND METHODS

Experimental Design and Maintenance

Polypropylene rectangular crates (60L) with 50 L level of water were used as experimental units, while genetically improved farm tilapia (GIFT) was used as the experimental fish. Advanced fingerlings of GIFT tilapia (200 fish) were bought from the Tilapia Breeding Center of the National Aquaculture Development Agency, Udawalawa, Sri Lanka. Before beginning the experiment, all fingerlings were acclimatized for a one-week period. After the acclimatization period, fish with an initial mean weight of 12.84 ± 0.53g was introduced into the cleaned tanks at a stocking density of 12 fish per tank.

There were three treatments, control (0 μgL^{-1}), Cd 50 (50 μgL^{-1}) and Cd 100 (100 μgL^{-1}). Each treatment with quadruplicates was randomly allocated into a complete randomized design, and the experiment lasted for six weeks. The highest Cd level was selected based on the maximum tolerance limit for the discharge of industrial wastewater into inland surface waters (100.0 $\mu g.L^{-1}$) recommended by the Board of Investment (BOI) Sri Lanka (Buhary 2015). Fish were fed with a commercially available diet two times per day to near satiety. During the experimental period, the number of dead fish in each tank was examined and recorded. Water quality parameters (Temperature. pH, DO and NH_3) were maintained at optimum levels throughout the experiment.

Growth Performance and Feed Utilization Parameters

The total length and body weight of the fish were obtained using a measuring board and a top-loading electronic balance respectively at the commencement of the experiment and every two weeks. Daily feed consumption was recorded, and the % Average Daily Gain (% ADG), % Specific Growth Rate (% SGR) and Feed Conversion Ratio (FCR) were calculated.

$$\%ADG = \frac{(Wt_2 - Wt_1)}{Wt_1(t_2 - t_1)} \times 100$$ **…….Eqn 01**

(Ricker 1979)

$$\%SGR = \frac{\ln(Wt_2) - \ln(Wt_1)}{t_2 - t_1} \times 100$$ **Eqn 02**

(Hopkins 1992)
Where,
Wt_1 is the mean weight at time t_1
Wt_2 is the mean weight at time t_2

$$FCR = \frac{feed\ intake\ in\ g}{increase\ in\ body\ weight\ in\ g}$$ **Eqn 03**

Cadmium Accumulation in Fish Tissues
Before commencing the experiment, fifteen fish with a mean body weight of 12.76 ± 0.73 g were sacrificed to obtain tissue samples of flesh, skin, gills, and liver for initial Cd analysis. At the end of the experiment, all fish from each treatment were sacrificed to obtain the above-mentioned tissue samples for Cd analysis. Fish were sacrificed and tissues were separated using sharp dissecting instruments. Separated tissue parts were subjected to digestion as the method described by Ujah I *et al.* (2017). Fish parts (flesh, skin, gills, and liver) were separately dried at 70 °C for 24 hours and dried samples were ground into fine particles using a ceramic mortar and pestle. Ground samples were weighed into crucibles which were washed in 10% HNO_3 and the prepared samples were ignited at 750 °C for 2 hours in a muffle furnace. Ash was then scraped into glass vials and crucibles were rinsed with 10 ml of HNO_3 acid. The vials were then capped and shaken thoroughly. The extract was diluted with up to 25.00 ml with 0.05 $mol.dm^{-3}$ HNO_3 acid. The diluted extracts were measured for Cd by atomic absorption spectrophotometer (iCE 3000 AAS, Thermo Scientific, Canada).

Hematological Parameters
Blood samples were taken from caudal vein puncture using a disposable syringe (1 ml) to analyze red blood cell count (RBC), white blood cell count (WBC), hematocrit (HCT) and packed cell volume (PCV). Blood samples were analyzed at the beginning and the end of the experiment after six weeks. Initial hematological analysis was performed using five fish while two fish per replicate were selected for final analysis. Red blood cells and white blood cells were counted with the aid of a Hemocytometer using the standard method. Hematocrit and the packed cell volume were taken using a micro-hematocrit reader.

Statistical Analysis
Statistical analysis was done using the IBM SPSS 25.0 software package. Data of all evaluated criteria were initially checked for normality. When normality assumptions were met, the means of all the parameters were compared by one-way analysis of variance (ANOVA) with a significance level of 5% ($p<0.05$). When ANOVA was found to be significant, a post hoc comparison of means was performed using Tukey's multiple range test. All values were presented as mean ± SD.

Ethical Statement
The scientific and ethical responsibility of the animal experiment belongs to the authors. (There was no committee established on the ethics in the faculty at the time when our experiment was conducted. However, fish were killed using MS222 anesthesia, and every effort was made to minimize suffering).

RESULTS AND DISCUSSION

Growth Performance and Feed Utilization Parameters

All evaluated growth and feed utilization efficiency parameters of fish were not affected by the treatment and significant differences were observed only in the survival rate of fish in different treatments. At the end of the experiment, the highest survival was observed in the control (100%) followed by CD50 and CD100 respectively (Table 1).

Results showed no significant differences among most of the growth parameters in CD50 and CD100 treatments compared to the control except for food conversion ratio (FCR). Besides heavy metal exposure, several other factors decide the growth of fish. The growth and body weight are a culmination of many biochemical phenomena (Almeida *et al.* 2001) and that biochemical changes can occur before the reduction in growth is observed (Miliou *et al.* 1998). According to Love (1970), weight is a non-specific measurement because it reflects not only changes in protein

content but also the degree of tissue hydration. Almeida *et al*. (2001) further indicated that the macromolecular content (various enzymes, glucose, glycogen, etc.) is sensitive to toxicant exposure and provides some perception about how the biochemistry of the fish was affected by cadmium hence these macromolecules could be used as bioindicators for toxicant effects on the fish since the food intake was not altered.

This experiment proved that even low concentrations of cadmium can cause increased mortality in comparison with the control. The observed high mortality may be explained by the inhibition of energy-yielding processes or interference with appetitive behavior (Miliou *et al.* 1998). As per Puvaneswari and Karuppasamy (2007), the mortality of cadmium-exposed fish was found to be time and concentration-dependent which may partly be due to the gradual accumulation of cadmium in the fish.

Cadmium Accumulation in Fish Tissues

The highest cadmium accumulation was observed in CD100 followed by CD50 and control. The highest accumulation was detected in the liver, gills, muscles, and skin respectively (Table 2).

There was no significant difference between the initial and the control for all tissues (muscle, skin, gill, and liver). Mean cadmium concentrations (Cd μgL^{-1} per g of tissue weight) in the muscles of the experimental fish showed a clear statistical difference among treatments ($P < 0.05$). According to the results, Cd100 has the highest Cd level (128.26 Cd μgL^{-1}) while the control has the lowest Cd level (3.80 Cd μgL^{-1}) among the tested treatments. Cd100 has a significantly higher level of mean cadmium level in the skin (105.06 Cd μgL^{-1}) and the lowest Cd level (2.23 Cd μgL^{-1}) is presented with the control despite the initial concentrations (Table 2).

Significant differences were also observed in mean cadmium concentration in liver tissue of different treatments. The highest accumulation level was observed in Cd100 (703.98 Cd μgL^{-1}) and the control shows the lowest level of cadmium accumulation level (7.71 Cd μgL^{-1}). In all treatments, the average concentrations of cadmium in gills were significantly different from each other ($P < 0.05$). Cd100 has the highest level of the Cd in gills (390.67 Cd μgL^{-1}) while the lowest Cd level was reported in the control (4.83 μgL^{-1}).

When considering the proximity to the toxicant of the various tissues analyzed, skin and gills are in direct contact with the toxicant medium. However, results showed considerable differences in cadmium accumulation be-

Table 1: The growth performances and feed utilization parameters of GIFT (Mean ± SD, n = 32)

Parameters	Treatments		
	Control	CD50	CD100
Initial total length (cm)	9.40 ± 0.06	9.10 ± 0.23	9.40 ± 0.47
Final total length (cm)	12.70 ± 0.15	12.80 ± 0.11	12.70 ± 0.13
Initial body weight (g)	12.89 ± 0.26	12.67 ± 0.88	12.95 ± 0.39
Final body weight (g)	32.23 ± 0.75	30.73 ± 0.96	31.24 ± 1.54
% ADG	3.58 ± 0.22	3.41 ± 0.35	3.32 ± 0.47
% SGR	2.18 ± 0.09	2.11 ± 0.14	2.09 ± 0.08
FCR	1.41 ± 0.03	1.37 ± 0.05	1.34 ± 0.03
% Survival	100.00 ± 0.0^{a}	87.50 ± 2.4^{b}	78.13 ± 1.9^{c}

Means with different superscripts in each row (a, b, c) are significantly different (P < 0.05) (ADG; average daily gain, SGR; specific growth rate, FCR; food conversion ratio)

tween gills and skin even though they come into direct contact with the ambient toxicant. Jayakumar and Vattapparumbil (2006) have stated that the physiological state of the tissue and structural and functional organization of these organs may be a probable reason for the observed difference in the metal accumulating capacity of gills and skin. They further mention that gills act as a primary site for cadmium accumulation in fish due to their external position and proximity to the ambient toxicant.

Highly branched structural organization of the gills and the resultant highly increased surface area, along with a large amount of water passing through the gill surface also make the gill a prime site for the accumulation of cadmium (Meyer *et al.* 1991). Besides, Meyer *et al.* (1991) further mentions that the highly vascular physiological state and the relatively small biomass when compared to their surface area also contribute to making the gill a primary site for cadmium accumulation.

The results of the present study show a far lesser rate of bioaccumulation of cadmium in the skin compared to the other tissues. As reported by Moore and Ramamoorthy (1984), cadmium undergoes multiple bonding in the body, forming stable complexes with a variety of organic compounds. Increased mucogenesis might result under the influence of toxicants with the formation of a mucous trap over the gills for the Cd^{2+} ions (Rajan and Banerjee 1991). This mucogenic activity of the body skin epithelium in fish is very high compared to the gills and increased mucogenesis may act an important role in avoiding the cadmium ions from entering the body. This coagulated mucus all over the body might be playing as a protective ion trap thus the accumulation rate of cadmium in fish skin is quite low (Hemalatha and Banerjee 1997).

Even though the liver does not come into direct contact with the toxicant, the same pattern of cadmium accumulation can be seen in the liver as that of the gills. The capacity to accumulate cadmium brought by blood from other parts of the body induces the production of the metal-binding protein, metallothionein, which is believed to play an important role against the toxic effects of heavy metals by binding them. That may be one of the main reasons attributed to the increased presence of cadmium in the liver (Bhattacharya *et al.* 1985). According to Kent (1998) the liver is involved in the detoxification and removal of toxic substances circulating in the bloodstream. For subsequent elimination, cadmium might be transported into the liver from other tissues, including gills and muscles. Such transportation might lead to higher rates of cadmium accumulation in the liver. The results of the present study showed that the cadmium levels were found to be higher in the liver followed by gill and muscle tissues in GIFT tilapia. Similar results were recorded in the study which is investigated by Çoğun et *al.* (2003) for the Nile tilapia (*Oreochromis niloticus*).

Among the four tissues investigated in the present study, the muscle also accumulated a low level of cadmium but higher than that accumulation level in the skin. There may be various reasons attributed to the lower rate of

Table 2: Cadmium accumulation in selected tissues of GIFT (Mean ± SD, n = 32)

Fish Tissue	Cadmium concentration (Cd μgL^{-1} per g of tissue weight)			
	Initial	**Control**	**Cd50**	**Cd100**
Muscle	0.85 ± 0.09^{c}	3.80 ± 1.01^{c}	16.33 ± 2.59^{b}	128.26 ± 9.14^{a}
Skin	0.30 ± 0.01^{c}	2.23 ± 0.59^{c}	10.99 ± 0.85^{b}	105.06 ± 1.92^{a}
Liver	1.74 ± 0.41^{c}	7.71 ± 1.64^{c}	37.75 ± 6.37^{b}	703.98 ± 12.22^{a}
Gill	1.29 ± 0.41^{c}	4.83 ± 0.82^{c}	31.77 ± 7.02^{b}	390.67 ± 15.72^{a}

Means with different superscripts in each row (a, b, c) are significantly different (P < 0.05)

cadmium accumulation in muscle. The muscle does not come into direct contact with the toxicant medium as it is covered externally by the skin contributing to preventing the penetrating of the toxicant (Jayakumar and Vattapparumbil 2006). Another reason may be the fact that even though the muscle is the most valued edible tissue, it is not an active site for detoxification. Therefore, the transport of cadmium to muscle from other tissues does not seem to arise (Jayakumar and Vattapparumbil 2006).

Cadmium accumulation in fish tissues may vary from species to species and depends on the exposure period. In Nile tilapia, the highest accumulation of cadmium has been reported in the kidney (Saðlamtýmur *et al.* 2004) while gills are the highest cadmium-accumulated tissue in *Cyprinus carpio* (Karaytug *et al.* 2007). However, heavy metals are rarely distributed uniformly within the tissues of fish and are accumulated by particular target organs (Jayakumar and Vattapparumbil 2006).

Hematological Parameters

In the present investigation, the most common hematological variables were measured including red blood cell count, white blood cell count, hematocrit, and packed cell volume. According to the results, at the end of the experimental period mean red blood cell count (RBC) declined in cadmium-exposed treatments compared to the control showing significant differences among treatments (Table 3). Among treatments, mean percent hematocrit (% HCT) and packed cell volume (% PCV) were not significantly different at the end of the experiment. In the present study, white blood cell count showed a clear statistical difference among treatments while CD100 had the lowest WBC compared to the control (Table 3).

Hematology can be considered an important index to the general health status of fish because it is often used to identify physiological changes in different stress conditions. As has been reported by Vinodhini and Narayanan (2009), there is a significant decrease in the RBCs of freshwater fish exposed to heavy metals. Due to the inhibition of erythropoiesis, the anaemic conditions were experienced in the fish to the chemical exposure (Nagarajan *et al*. 2014) and this was revealed in the present study by the reduction in RBC number.

A similar pattern of RBC reduction had been observed by Pereira *et al*. (2016) in *Rhamdia quelen*, Houston and Keen (1998) in goldfish (*Carassius auratus*) and Lowe-Jinde and Niimi (1986) in rainbow trout (*Salmo gairdneri*) exposed to cadmium. Lowe-Jinde and Niimi (1986) also stated that this RBC reduction might be due to a decrease in the synthesis or release of red blood cells into the circulation. Furthermore, they suggested that cadmium caused a reduction in erythropoiesis and impeded the formation of red blood cells.

White blood cell count remained reduced among cadmium-exposed treatments until the end of the experiment. Similar changes were observed by Witeska (2005) and it might be

Table 3: Various hematological parameters (Mean ± SD, n = 32) of GIFT tilapia in different Cd concentrations

Hematological parameters	Initial	Treatments		
		Control	CD50	CD100
RBC ($\times 10^6$ mm^{-3})	1.20 ± 0.08^{c}	2.21 ± 0.07^{a}	1.50 ± 0.05^{b}	1.28 ± 0.04^{c}
WBC ($\times 10^3$ mm^{-3})	244.33 ± 4.51^{c}	468.00 ± 6.61^{a}	293.11 ± 3.62^{b}	205.19 ± 8.94^{d}
HCT (%)	12.63 ± 3.26^{b}	24.53 ± 2.92^{a}	22.71 ± 1.88^{a}	22.03 ± 0.85^{a}
PCV (%)	14.12 ± 3.39^{b}	25.92 ± 2.70^{a}	24.02 ± 1.84^{a}	23.41 ± 0.88^{a}

Means with different superscripts in each row (a, b, c, d) are significantly different ($P < 0.05$) (RBC; red blood count, WBC; white blood count, HCT; hematocrit, PCV; packed cell volume)

due to the secretion of cortisol during the stress reaction which shortens the life span of lymphocytes and promotes their apoptosis (Weyts *et al*. 1998; Adeyemo 2007) and reduces their proliferation (Espelid *et al*. 1996).

CONCLUSIONS

The results of this study revealed that although the growth performance of experimental fish is not affected by cadmium exposure, survival is reduced with increasing the cadmium concentration while significantly affecting the bioaccumulation level and hematological parameters. Therefore, it can be suggested that there is a significant effect of the chronic exposure of cadmium on the bioaccumulation and hematological parameters of the GIFT tilapia even at the BOI recommended concentration.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTION

WS and JMSN conceptualized and designed the study. WS performed the experiment and sample analyses. and SS supervised the experiment. WS, JMSN and SS analyzed and interpret the data. WS drafted the paper with input from all authors. All authors discussed the results and commented on the manuscript.

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