GROWTH AND SURVIVAL ON EXPOSURE TO LAMBDA CYHALOTHRIN AND AFLATOXINS OF DIETARY FISH FROM SELECTED AQUATIC SOURCES IN KENYA

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Abstract

Toxicants cause a wide range of concerns in the health of fish. The specific objectives of the study were determine aberration of growth and survival as an iron indicator on exposure to lambda cyhalothrin and aflatoxins in dietary fish from selected aquatic sources in Kenya. The total wet weight was measured to the nearest 0.1g with an electronic weighing balance (Scanvaegt salter, model 323; Avery Weigh-Tronx Ltd, West Midlands, UK)Gen Stat Version 12, SPSS Version 21 and Excel 2010 were used in data analysis. The completely randomized design was adopted and Turkeys' to separate the means having set the significance levels at $p<0.05$. The specific growth rate in length was lower in aflatoxin treated fish in bred fish. Garipenus Spp had the lowest the specific growth rate of 10.53 % on treatment with aflatoxin as compared to lambda cyhalothrin. In Oreochromis Spp the treatments affected the SGR equally giving a lower SGR of 24.6% compared to the control. The natural occurring toxins negatively affect growth and therefore relevant stakeholders be keen to prevent contamination from farm to fork.

Keywords: Dietary. Fish. Aberration. indicator . Iron, Growth. Aflatoxins. Lambda cyhalothrin

Introduction

In research done it was shown that aflatoxin treatments that the daily feed ration given to the fish was not completely consumed, an indication of the decreased appetite of fish or perhaps due to unpalatable feed. Decreased growth and appetite were observed by Royes *et al.* (2002) when tilapia was fed with diets containing 1,800 ppb of aflatoxin for 75 days. Chavez *et al*. (1994) reported that *O. niloticus* fingerlings were able to tolerate the immediate effect of aflatoxin but later the fish developed external and internal abnormalities.

Furthermore, Nile tilapia that were fed diets with 10mg AFB/kg for 8 weeks had 90% reduction in growth rates compared to 24% growth reduction in channel catfish fed the same amount (Jantrarotai & Lovell, 1990; Tuan *et al*., 2002).

According to Tuan *et al*. (2002) the intensity of adverse effects induced in Nile tilapia, by AFB1 dietary administration for 8 weeks, increased proportionally along with the aflatoxin concentration ingested: fish fed diets containing 2.25mg AFB/kg had changes in growth rate, whereas 10mg AFB/kg produced hepatic lesions, and 100mg AFB/kg caused severe hepatic necrosis and 60% mortality, as reviewed by Gallagher and Eaton (1995). Average body weight and FCR of Beluga were significantly affected by 75 and 100ppb AFB1 in diet but not by 50ppb or less. Although, there was significant differences between FCR in treatment two diets with 75ppb AFB1 with control diet and no statistical different observed between other treatments, but functional differences could have economical effects on large farm production. There was no significant difference in SGR between experimental treatments and control after three months. SGR has a close relation with weight gain, but it was not affected.

Cha´vez-Sa´nchez *et al*. (1994) reported similar findings for Nile tilapia; growth was not affected by the 0.94mg/kg diet, but was reduced by diets containing 1.88mg AFB/kg or higher. However, these authors reported that FCR was not affected by AFB levels as high as 30mg/kg. An inverse relation between AFB concentration and growth rate of Nile tilapia was reported by El-Banna *et al*. (1992)**.**

Toxicity tests conducted at levels of lambda cyhalothrin residues measured in water or sediment indicate potential for effects on aquatic organisms including fish and amphipods as stated in various studies (Amweg et al. 2005, Cavas and Ergene-Gozukara 2003; Gu et al. 2007; Heckmann and Friberg 2005; Lawler et al. 2007; Maund et al. 1998;Van Wijngaarden et al. 2005). Concerns have therefore been raised about the widespread use of lambda cyhalothrin that it causes growth retardation in fish.

Since fishes are important sources of proteins, minerals and lipids for humans and domestic animals, so health of fishes is very important for human beings. Fish like other aquatic organisms may be exposed to a great range of insecticides during the course of their life cycle. In fish, different insecticides can be absorbed through gills, skin or alimentary ducts (Schlenk, 2005; Banaee et al., 2011; Banaee, 2012). Fishes are particularly sensitive to environmental contamination of water. Hence, pollutants such as insecticides may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes (Banaee et al., 2011). So, the effects of insecticides on fishes are of great concern. Aflatoxin is a hepatotoxic, carcinogenic, and immunosuppressive, anti nutritional contaminant of many staple food commodities. Contamination may develop as a result of fungal action before and during harvest and also during storage (Williams et al, 2004).

Contamination of food, feed and agricultural commodities by aflatoxins poses enormous economic and serious health concerns. The toxicological effects of aflatoxins are that they are highly carcinogenic and can directly influence the structure of DNA. The resulting genetic defects can lead to fetal malformations and miscarriages; aflatoxins are also known to suppress immune systems (Razzaghi-Abyaneh *et al.*, 2015).

Two prospective cohort studies in the Faroe Islands and New Zealand have shown that prenatal and early postnatal exposure from seafood is associated with cognitive deficits in children, including attention, perceptual deficits, select language, and general cognitive deficits (Grandjean *et al* ,1998 and Crump *et al* 1998).

A number of populations are at particular risk of persistent organic pollutants (POPs) exposure, including people whose diets include large amounts of fish, shellfish, or wild foods that are high in fat and locally number of populations are at particular risk of POPs exposure, including people whose diets include large amounts of fish, shellfish, or wild foods that are high in fat and locally obtained. For example, indigenous people may be particularly at risk because they observe cultural and spiritual traditions related to their diet. In addition, sensitive populations, such as children, the elderly, and those with suppressed immune systems, are typically more susceptible to many kinds of pollutants, including POPs. Because POPs have been linked to reproductive impairments, men and women of child-bearing age may also be at risk. Due to biomagnifications and bio concentrations, small releases of POPs can have significant impacts.

The effects can be acute or chronic causing morbidities, mortalities, disability or death. It is therefore important to ensure fish quality to avoid problems arising as a result of contamination.

Previous research work in Kenya have conducted studies on the effects of toxicants on the other aspects of health, but not taken a step further to conduct research on the influence of essential nutrients like iron. The natural and synthetic toxicants in fish may be causing significant changes in the iron levels due to metabolic disruption, and yet no research has specifically checked for the availability of iron. The knowledge gap on nutrient toxin interaction was addressed as findings from this study therefore linked toxicants and availability of iron in fish as a heme food source. Results from this study generalized and shared with food scientists, food toxicologists, medical personnel, agriculturalists and the community at large on the magnitude on iron availability in fish on exposure to natural and synthetic toxicants in fish a heme food sources. Previous studies have considered the implications of nutrient toxicant interactions but findings from this study encouraged nutritionists to consider the importance of environmental exposures to their study populations and their research questions. Furthermore, it encouraged the involvement of nutritionists in the design of high quality, rigorous studies of nutritional assessment and interventions in populations exposed to environmental chemicals. As a growing field, the intersection between nutritional science and toxicology benefited from the expertise of nutritionists.

Materials and methods

Study design and setting

For fish bred at Sagana Experimental study design was adopted where manipulation of independent variables to determine their effect on a dependent variable. A completely randomized block design was adopted. For Fish from River Nyando were collected at three different points . A Cross Sectional study design was also adopted where fish samples were collected from River Nyando for laboratory analysis. The independent variables were the treatments and the dependant variable being iron levels. (Kothari, 2004).

Study species and sample size

For fish bred at Sagana Identification of *Oreochromis niloticus* and *Clarias gariepinus* species was done by the Kenya Marine and Fisheries Research Institute (KMFRI) staff. Nine Hundred (900) fish, 450 of each species were bred in the lab between January – August 2015. Sample size calculation for fish in bred at Sagana

$2\ SD^2\ (Z^t + Z^x)^2\ /d^2$

SD = Standard Deviation from Pilot Study

Z^t = Z value from the Z table

Z^x = type 1 error of 5% at 80% interval

D=effect size, difference in means (iron in mg) (Jaykaran and Kantharia, 2013).

$=2(1)^2\ (1.96+0.842)^2/3.5$

$= 4.48$

=5 fish per tank

Fish from River Nyando

$2\ SD^2\ (Z^t + Z^x)^2\ /d^2$

$=2(1)^2\ (1.96+0.842)^2/3.5$

$=2(1)^2\ (1.96+0.842)^2/0.145$

$= 108.29$

=108 fishes

Sampling Techniques

For fish bred at Sagana, there are about thirty five species of fish commonly consumed in Kenya were written down, and the two fish species were selected randomly. For the fish bred in aquarium in the lab, all the fish sampled were used for the experiment. The fish species used were *Oreochromis niloticus* and *Clarias gariepinus* and were cultured in the lab for twelve weeks after demineralization of water. The samples were extracted immediately and analyzed for iron levels. This was after treatment with aflatoxins in feed prepared and lambda cyhalothrin introduced in water and parameters measured when fish were at various ages. For fish from River Nyando the sampling procedure for fish species collection was the same as in fish bred in Sagana. The fish were selected at three different sites along River Nyando. For each site fish were collected by means of gill net mesh size. The nets were deployed from early morning, and checked two hourly until the required fish quotas were reached. This minimized the amount of time fish spent in the net in order to reduce any imposed stress. Only living fish size 10-60g were selected, kept in a 100L plastic tank and immediately transported to laboratory for sample collection and necropsy analysis. A total of 105 fish specimens were collected from River Nyando and length and weight measurements taken

Growth monitoring

For both fish samples collected from fish bred at Kenya Marine and Fisheries Industries (KMFRI) Sagana and from River Nyando the laboratory sites and procedures were similar. At the laboratory, fish were kept in large holding tanks filled with water from each site to minimize stress. Fish were weighed and total length (TL in cm) was measured at respective fish collection points. In addition, the sex of each fish specimen was also recorded. Total length was measured to the nearest 0.1cm, using a measuring board. The total wet weight was measured to the nearest 0.1g with an electronic weighing balance (Scanvaegt salter, model 323; Avery Weigh-Tronx Ltd, West Midlands, UK)

Detection of Lambda Cyhalothrin was conducted in water from River Nyando. Sample analysis was done using Varian CP 3800 Gas Chromatograph equipped with Electron Capture Detector. Separation was done using BPX 5 capillary column of dimensions 30 m x0.25 mm x 0.25 μm film thickness. Confirmatory analysis was done using BPX35 capillary column of dimensions 50 m x 0.25 mm x 0.25 μm film thickness. A temperature program

was used starting from 90 0C (with hold time of 3 minutes), increased to 215 0C at 8 0C/min (with hold time of 25 min), then increased to 270 0C at 5 0C/min (withhold time of 5.37 min), and finally ramped to 275 0C at 5 0C/min (with hold time of 18.63min). The carrier gas was high purity helium (99.9995%) with white spot nitrogen as the makeup gas. Quantification followed external calibration method using high purity pesticide reference standards mixture obtained from Ultra Scientific USA. Quality control and Quality assurance was ensured by all sampling, extraction and analysis being done in triplicate to allow verification detected PoPs residues. The samples were spiked with the insecticide during extraction and analysis to minimize errors due to detector fluctuations. Recovery tests were also carried out using the reference pesticide standards to determine performance of the methodology. Quantification of LCH was carried out using high purity organic pollutants.

Data management and analysis

Data Reporting

For both fish obtained from Sagana and from River Nyando, data included length and weight. For fish from River Nyando, Lambda Cyhalothrin and aflatoxin were recorded from the water samples. (Rognerud *et al*.2002 and Rosseland *et al*. 2007).

Data analysis

The independent variables were the treatments aflatoxins and Lambda cyhalothrin. The dependent variables were length and weight. The data was entered into a computer and analyzed using the excel spreadsheets as data base and GENSTAT version 12 analysis for means, frequencies and cross tabulations. For fish obtained from River Nyando, SPSS version 21 was used for data analysis. One way ANOVA was carried out to determine differences in means of length and weight, lambda cyhalothrin and aflatoxins. Specific growth rate was calculated in fish bred in Sagana. Post –hoc HSD (turkey) (was used for Post-hoc discrimination between means. In all statistical tests 5% significance level was applied.

Ethical Considerations

All experiments with fish were conducted in accordance with national and institutional guidelines for protection of animal welfare (Prevention of Cruelty to animals Act, Cap 360 of the Laws of Kenya). Authority to conduct research was sought from the Fisheries department of Kenya and from the various sites, KMFRI Sagana.

Results

Comparing the significance of treatments on weight of *Oreochromis spp* and *garipenus* spp in fish obtained from River Nyando and cultured fish. Weight in grams (g).

The type of treatment had no significant influence on weight of the fish ($p<0.05$) as shown in Table 1 below in fish from all environments.

Table 1: Comparison of means showing the differences in length and weight of *Oreochromis spp* and *garipenus* spp in fish from River Nyando and cultured fish on exposure to Lambda Cyhalothrin versus Aflatoxins . Weight in grams (g).

Condition	Aflatoxin	S.E.M	Lambda Cyhalothrin	S.E.M	P value
Sagana (Length)	7.71±0.76	0.72	8.83±1.49	0.35	0.014
RiverNyando(Length)	ND	ND	21.94±6.04	0.581	0.420
Sagana(Weight)	5.87±3.34	0.79	7.54±2.41	0.57	0.249
RiverNyando(Weight)	ND	ND	47.17± 17.69	1.702	0.292

H_o There is no significant effect of growth on exposure to lambda cyhalothrin versus aflatoxins on growth in dietary fish in selected environments in Kenya.

The means did not affect most of the growth parameters significantly hence the null hypothesis was accepted.

Effect of toxicant exposure to growth

Fish from River Nyando were heavier than fish bred from Sagana (Table 2).

Table 2: Comparison of means on weight and length of *Oreochromis spp* and *garipenus* spp in fish from River Nyando cultured fish. Length in centimeters.

Environment	Species1	S.E.M	Species2	SEM
Sagana(Length)	8.70±0.85	0.16	7.72±1.21	0.28
RiverNyando(Length)	27.39±4.57	0.58	18.05±1.34	0.43
Sagana(Weight)	4.75±1.70	0.33	8.83±0.51	2.68
RiverNyando(Weight)	63.07±14.68	2.19	35.17±8.52	1.07

Growth rate of fish

Results from this study as shown from Table 3 indicate that in *Garipenus Spp* had the lowest the specific growth rate of 10.53 % on treatment with aflatoxin as compared to lambda cyhalothrin. In *Oreochromis* Spp the treatments affected the SGR equally giving a lower SGR of 24.6% compared to the control.

Table 3: Change in Length of *Oreochromis* spp and *Garipeunus* spp in fish bred in Sagana

Treatment	Aflatoxin	Control	Lambda Cyhalothrin
Initial Length Garipenus *spp*	7.6	8.4	7.7
Final Length	8.4	10.7	9.1
Change in Length	0.8	2.3	1.4
SGR	**10.53**	**27.38**	**18.18**
Initial Length Oreochromis *spp*	6.5	6.8	6.9
Final Length	8.6	8.9	8.6
Change in Length	2.12	2.1	1.7
SGR	**24.6**	**30.8**	**24.6**

Treatment	Aflatoxin	Control	Lambda Cyhalothrin
Initial Weight Garipenus *spp*	3.0	3.2	4.3
Final Length	3.6	5.4	6.3
ChangeinWeight	0.6	2.2	2.0
SGR%	**20**	**68.75**	**46.5**
Initial Weight Oreochromis	5.1	5.9	6.7
Final Weight	13.0	11.9	11.5
Changein Weight	7.1	6.8	4.8
SGR	**120.3**	**133.3**	**71.6**

Table 4: Change in weight of *Oreochromis* spp and *Gariepinus* spp in cultured fish

From presentation of results in Figure 1 the *Garipenus* spp had the lowest SGR of 20% on treatment with aflatoxins as opposed to Oreochromis spp which had had the Highest SGR.

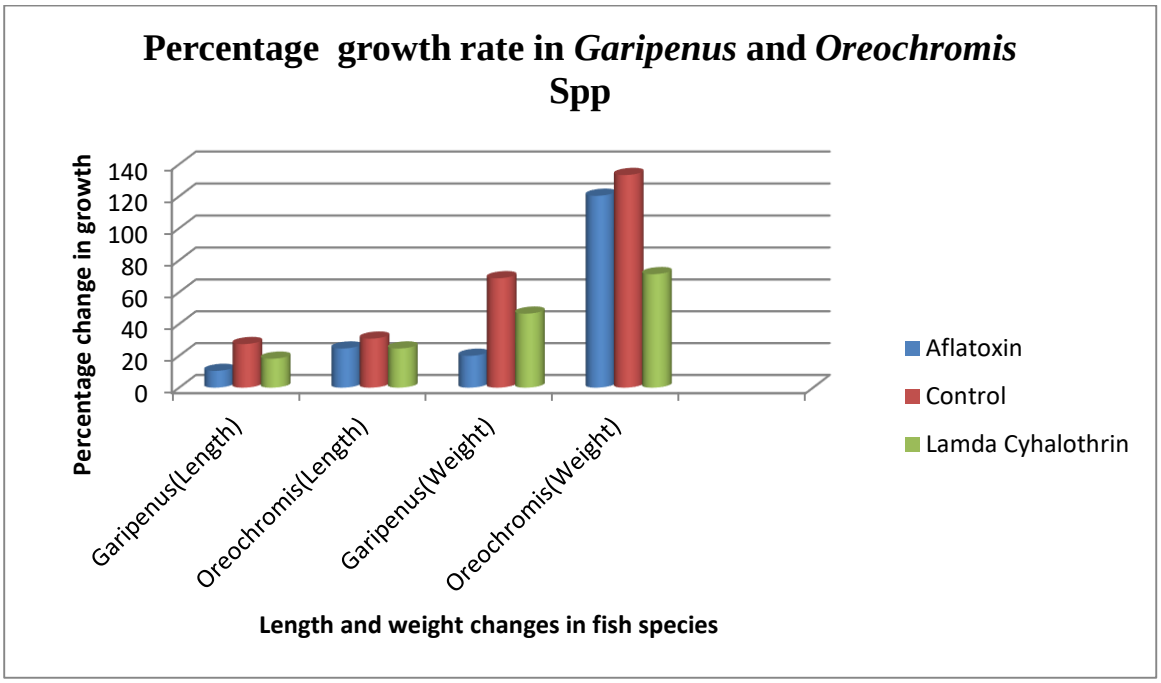

Figure 1: Bar Graph showing a comparison of growth in fish species

Table 5: Kaplan Meir Survival on exposure to Lambda Cyhalothrin Versus Aflatoxin in *Clarias gariepinus* and *Oreochromis niloticus* in cultured fish.

Group	Species Population	Time
Aflatoxin	75%	4.2
	50%	3.5
	25%	3.2
	95%	(3.3,4.2)
Control	75%	7.0
	50%	6.5
	25%	6.3
	95%	(6.3,6.9)
Lambda Cyhalothrin	75%	5.3
	50%	4.7
	25%	4.2
	95%	(4.2,4.9)

4.4. 4. Survival of fish

Results as indicated on Table 5 indicate the fish treated with aflatoxin in group 1,which is the aflatoxin exposure have the highest morbidities with 25% of the fishes at 3.2 months being alive. The group 2 which is the control have the lowest morbidities with 25% of fish in that group being alive at 6.3 months.

Table 4.6: Test statistics for equality and survival on treatment with Lambda Cyhalothrin and Aflatoxin in *Clarias gariepinus* and *Oreochromis niloticus* in cultured fish.

Parameter	Statistic	Probability
Log rank	**74.889**	**0.001**
Wilcoxon(Browslow)	**57.718**	**0.001**
Tarone-Ware	**65.757**	**0.001**
Wicoxon-Peto Prentice	**57.763**	**0.01**

From the results table 6 indicators on survival are all significant.

Discussion

Iron contributes for growth and well being in fish. In this study, Lambda cyhalothrin treatment gave the lowest increase in weight and length. Aflatoxin also had a lower growth rate as compared to the control. This is consistent with other studies which revealed significantly reduced growth rate among species with body weight gain in aflatoxin treated fishes showed significant decrease($p>0.05$) as compared to control or fishes given feed I or mold free feed. The average body length gain and percent body length gain was also significantly lower($p>0.05$) in fishes fed with feed II, II and III as compared to fishes given feed I or mold free feed (Caguan,2007). Results from this present study further agree with the findings of Jantrarotai and lovel (1990) in *Oreochromis aureaus*, Roges *et al*. (2002) in *Oreochromis nilotius*, Nguyen *et al*. (2002) in Juvenile Nile Tilapia and Zaki *et al*. (2012) in *Clarius lazera*. Joner *et al*. (2000) reported that aflatoxin reacts negatively with different cell protein which leads to inhibition of carbohydrate and lipid metabolism and protein synthesis. So the decrease in growth rate in experimental fish may be due to disturbance in metabolic process of carbohydrates, lipids and proteins by aflatoxin.

Cheeke and shull (1985) reported that aflatoxin causes loss of appetite. Thus the decrease in average weight gain and body length increase may also be due to loss of appetite. Also it might be due to utilization of glutathione enzymes for detoxification process under the condition of Stress (Devegowda *et al*. (1998). Glutathione enzymes are partly consist of methionine and cystein and hence this process of detoxification decreases availability of methionine resulting in poor growth in the fish.

Further studies are in agreement with the present study on the Nile tilapia (*Oreochromis niloticus*) showed reduced growth rates when tilapia were fed diets containing 1.8 milligrams (mg) of AFB1 per one kilogram (kg) of feed for 75 days. In addition, tissue abnormality or lesions in the livers of these tilapia showed the beginnings of cancer development. Another study (Tuan et al. 2002) tested effects of varying concentrations of AFB1 on 2.7-gram Nile tilapia

Fish fed diets that contained 2.5, 10, or 100 mg AFB1 per kg of feed for 8 weeks had reduced weight gain and reduced red blood cell counts.

Accumulating evidence suggests that anthropogenic discharges of chemicals and complex mixtures are capable of eliciting endocrine disrupting effects that adversely affect the health

of humans and wildlife. Current studies are finding that numerous species experience compromised reproductive fitness and increases in hormone-dependent cancers. Researchers are also concerned that these endocrine disruptors can have other effects such as altered immunity and decreased disease resistance, especially during embryonic and fetal development. These chemicals can alter hormone metabolism resulting in elevated levels of androgens, which may lead to pseudohermaphrodism, developmental abnormalities, and reproductive impairment. Chemicals known or suspected to cause these effects and act as synthetic estrogens include organochlorine pesticides, polychlorinated biphenyls, dioxins and other synthetic chemicals. This physiological imbalance affects growth of fish as well.

Survival percentage decreased with increase in aflatoxin containing feed. The fishes which were given aflatoxin free diet or feed I showed hundred percent survival whereas minimum survival ie forty four percent was found in those fishes which were fed with feed I. Thus the present findings are in agreement with those of Caguan *et al*. (2004).The decreased survival percentage was probably due to impaired liver function, loss of appetite and decreased immunity as a result of aflatoxin.

Conclusions

Growth rate in length and weight are influenced by iron. In this study, the treatments had no influence on the growth. Growth is not dependent on iron levels alone but on other factors as well. Growth rate was no treatment sensitive but species sensitive.

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