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EFFECT OF AFLATOXIN B_1-CONTAMINATED FEED ON GROWTH AND VITAL ORGANS OF ADVANCE FRY OF, *Catla catla*

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ABSTRACT

The study was conducted for the period of 90 days to investigate the effect of Aflatoxin $B_1$when present in feed on the growth and histology of advance fry of *Catlacatla*. The fish was rerered in glass aquaria in Fish Hatchery Complex of the Department. There were 5 treatments and a control with two replicates in each. 180 advance fry were randomly stocked in each glass aquaria. 15 fish per aquaria containing 90 liter fresh clean tubewell water. Five fish were then randomly collected from each aquaria weighed and measured. The prepared feed was divided into 6 equal parts. Part 1 was as control while remaining 5 parts received Aflatoxin in the order of 10, 20, 30, 40 and 50 ppb termed as T_1, T_2, T_3, T_4 and T_5. Water quality was monitored on daily basis. At the end of the experiment, liver, kidney and intestine were removed for histopathological studies. Results showed that the body weight of advance fry *Catlacatla* was the highest in control group as compared to aflatoxins treated aquariums. The growth of aflatoxicosed fish was significantly different and lower than control ($p< 0.05$). Specific growth rate was higher in control aquarium, lowest was in T5 where 50 ppb aflatoxins were applied. Feed conversion ratio was highest in T5 as compared to control. The survival rate was 100% upto T3 while in T4 and T5 was lowest. Weight gain and food conversion ratio (FCR) varied significantly ($P<0.05$) between control and treatments with diets contaminated with 10 ppb to 50 ppb (AFB1/kg after 90 days). Several histological alterations were recognized in the liver of the fish examined and these were chronic manifestations. Collapsed liver were found in dead fish in T4 and T5. The liver of fishes in T2, T3 and T4 had extensive necrosis, acute cellular swelling or ballooning necrosis, chronic granulomatous inflammation, loss of color where the necrotic tissue becomes paler than the surrounding normal tissue. It is concluded that liver was the principal target organ for aflatoxins. After the invasion of aflatoxins into the liver, lipids infiltrate hepatocytes and leads to necrosis or liver cell death.

Key words: Aflatoxin B_1, contaminated feed, growth, vital organs, C*atla catla* fry.

INTRODUCTION

The fresh water fish, *Catlacatla* is one of the valuable Indian major carps that is being cultured extensively in polyculture or composite culture system with other Indian and Chinese major carps. A great deal of information is available on its taxonomy, ecology, abundance, culture practices, production methods, but toxicity of Aflatoxin B_1 on its growth, survival and histopathology is least explored component especially in Pakistan.

Gradual expansion in global aquaculture is increasing the demand for aquaculture feed, which is the prime input in fish cultural practices. Generally, the selection of feed ingredients for any production system depends upon its nutritional value and costs. Protein is the vital and expensive nutrient of formulated fish feeds (De Silva *et al*., 1989). Both the quality and quantity of protein in fish feed is of paramount importance in promoting fish growth for achieving marketable size of fish within a limited time period.Fish meal still is used globally as animal protein source in formulated fish feeds but its rising cost, limited production, uncertain availability, adulteration and variation in quality has forced aquaculturists to look for alternatives (Prabjeet *et al*., 2011;FAO 2004; Lunger *et al*., 2007). Therefore it will be necessary to replace this ingredient partially or totally with comparatively cheaper and easily available ingredients to make aquaculture operations viable and sustainable (Prabjeet *et al*., 2011); plant ingredients can be quoted as an example.Plant ingredients though cheaper but carry several problems one of them is Aflatoxin toxicity if feed or its ingredients are stored at high temperature and humidity. Aflatoxins are considered the most important mycotoxins, which are carcinogenic, mutagenic and teratogenic. These toxins are poisonousby products of the moulds *Aspergillusflavus* and *Aspergillusparasiticus*, and are important contaminants of certain foods and animal feeds (Farr *et al*., 1989). In aquatic animals, Aflatoxin can cause abnormalities such as poor growth, physiological disorders and histological changes that result in declined yield. Aflatoxins exert a substantial impact on the fish and shrimp farming production, causing disease with high mortality and a gradual decline of reared fish stock quality, thus representing a significant problem in aquaculture systems. The objective of these studies was to investigate

the effect of Aflatoxin B_1 when present in feed on the growth and histology of advance fry of *Catlacatla*.

MATERIALS AND METHODS

Experimental Station and Test Species: The experiment was conducted in glass aquaria in Fish Hatchery Complex at Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences Ravi Campus Pattoki.

Catla catla advance frywere used as experimental animals and studies were conducted for the period of three months.

Experimental Design: Completely randomized design was used to plan and manage current studies.There were 5 treatments and a control with two replicates in each. 180 advance fry were randomly stocked in each glass aquaria 15 fish per aquaria containing 90 liter fresh clean tubewell water. 12 glass aquaria were then randomly allotted and equally divided into 5 treatments and one control group. Five fish were then randomly collected from each aquaria weighed and measured.

Chemical Analysis of Feed Ingredients and preparation of feeds: Feed ingredients such as fish meal, maize gluten meal, soya bean meal, sunflower meal, rice polish and molasses were analyzed for the presence or absence of toxins by qualitative and quantitative methods. Moisture, crude protein and crude fiber levels in the feed and feed ingredients were determined following AOAC (2005). 30% crude protein containing feed was formulated and prepared in bulk by taking and combining the required quantity of the following feed ingredients in the proportions given against each.

Table 1: Feed formulations for advance fry *Catlacatla*

Name of Ingredient	30% Crude Protein		
	%Protein present in feed ingredient	Contribution in feed formula %	Amount of Ingredients Gm
Fish meal	50%	5	28
Maize gluten meal	30%	9	20
Soya bean meal	45%	9	20
Sunflower meal	30%	5	20
Rice polish	12%	2	10
Molasses	-	-	2
Total %		30	100

The prepared feed was then divided into 6 equal parts. Part 1 was kept free of aflatoxins and it served as control. The remaining 5 parts receivedAflatoxinin the order of 10, 20, 30, 40 and 50 ppb hereafter termed as T_1, T_2, T_3, T_4 and T_5. Aflatoxin was grown in Quality Operation Lab (IBBT) Istitute of Biochemistry and Biotechnology (University of Veterinary and Animal Sciences Lahore) checked for its concentration and purity and then mixed in various batches of feed as per requirement of the experimental treatments. All the fishes in various groups were regularly and equally fed at the rate of 5% of their body weight daily. Response to feed was measured in growth increments and physical damages to the vital organs were ascertained by the extent of histopathological changes.

Water quality parameters: Dissolved oxygen and temperature were monitored daily, ammonia, nitrates, phosphates, pH on weekly basis while hardness, chlorides, total alkalinity, total suspended solids on fortnightly basis.

Determination of body composition of fish: At the end of the experimental trial, all the fish was harvested weighed and measured. Some were dissected and their liver, kidney and intestine were removed and preserved in appropriate fixative for histopathological studies. Representaive samples of five fish were dried and ground for determination of crude protein, crude fat, ash, dry matter and moisture contents following AOAC (2005).

Histopathology

Dissection of fish: Advance fry were dissected after 90 days of feeding trial in the laboratory of Aquaculture and Fisheries Department Pattoki. Animals were placed in polythene bags. A vertical incision was made extending from the anal fin towards pectoral fin. The body was cut opened and kidneys, liver and intestine were lifted with the help of the forceps and then carefully placed in tissue cassettes with date, group and dose written on them and preserved in 10% formalin containing jars.

Tissue Processing: The processing of tissues for histopathology were carried out in the Pathology laboratory of University of Veterinary and Animal Sciences Lahore.

The tissue processing schedule of histological techniques was as follows:

Samples were run in different grades i.e in running water for one hour and for two hours each in all grades i.e 70% alcohol, 80% alcohol, 90% alcohol, 100% alcohol, again 100% alcohol, xylene and alcohol, xylene I , xylene II , wax I and wax II .

Cutting of Paraffin Sections: Cutting of fish tissues for histopthological studies was carried out in Quality Operation Lab (IBBT) Istitute of Biochemistry and Biotechnology (University of Veterinary and Animal Sciences Lahore). The process adopted for section cutting was as follows:

Water bath was filled with ordinary tap water and the water was kept at 10º C temperature. The tissue blocks were trimmed to size, keeping upper and lower edges parallel. The block was fixed in the rotary microtome and the angle of the blade was appropriately adjusted and blocks were cut at 4µ; ribbons of the cut sections were transferred to water bath spread them at 50 degrees. The ribbon was split into separate sections which were picked on the surface of albumenized and labeled glass slides. The slides were then dried in incubator for 15 minutes at 65 º C and then stained stained in Hematoxylin and Eosin.

Hematoxylin and Eosin Staining (Harris's method): Slides were placed vertically in incubator cum oven (Memmert) for 20 min at 60 °C for de waxing. The slides were then cleared in two changes, two minutes each, of xylene; one minute each, of alcohols. i.e 100% alcohol and 70% alcohol. The slides were neutralized by immersing in distilled water for two minutes. Slides were stained in Harris's Haematoxylin (sigma) for four minutes followed by blotting on filter paper. The slides were rinsed in running tap water for one minute. The slides were dipped in 1% acid alcohol for one second, followd again by washing in running tap water for one minute. The slides were neutralized in 1% ammonical water for one minute, again followed by rinsing in running tap water for one minute. The slides were immersed in 70% alcohol for one minute and were counter stained in Eosin solution (Fluka) for four minutes. Finally the slides were dehydrated by passing through ascending series of alcohol for ten seconds each clearing in xylene, one minute, and mounting using DPX mount (Di-N-Butyle Phthalate in xylene).

Statistical Analysis: Data were subjected to Analysis of Variance (ANOVA) to find out the significance level among various treatments. Differences among means were then distinguished by Duncan's Multiple Range Test. SAS (Statistical Analysisfor the Social Sciences) package for windows version 9.2 was used for all statistical tests. Significance level was set at $P < 0.05$.

RESULTS

Average Body weight of Advance fry *Catlacatla*: Average weight of fish stocked in aquaria was 2.51 gram in control group while it was almost uniform in all the other treatments which was2.05to 2.40 gram. Fish was reared on the same diet with variations in the concentrations of AflatoxinB1 which were 10, 20, 30, 40 and 50 ppb.

After fifteen days of rearing major increments were observed in control group, then T_1, T_2, T_3, T_4 and the lowest were in T_5. When these increments were compared over the time periodlapsed the growth remained uniform from the start to the end of the experiment. Maximum growth was observed in the control group and minimum in T_5. Growth in the remaining treatments fell in between these ranges. When the growth increments were compared among different treatments, differences remained insignificant from T_2 to T_5. However, the fish in control and T_1 grew equally and significantly higher than all the other treatments. The control remained at the top while fish in T_5 grew the least (Table 2).

Average Body length of advance fry*Catlacatla*: Similar trend was observed in length increments which were almost similar between control and T_1 however, differences were not so significant but still higher than all the other treatments. Lowest length increments were observed in T_5 but it did not show any relationship with the time lapse. Similarly the length increments in other treatments specifically in T_3 to T_4were the same. It was interesting to note that the fish grew equally right from the first fortnight till the last fortnight. However, in the case of control, T_1 and T_2 trend was rather different. Though there was not much variation in length increments among the fortnights but fish in control group gained slightly more length than T_1 and T_2. It will be worth mentioning that length increments in control, $T_1$and T_2were significantly higher than rest of the increments(Table 3).

Water Quality Parameters: All the water quality parameters viz: water temperature, dissolved oxygen, pH, alkalinity, total suspended solids, hardness, nitrate, ammonia, chloride remained same and with the acceptable ranges suitable for fish culture except dissolved oxygen, which was the highest in control group(5.58±0.53) but in the remaining dietary treatments though it was significantly lower than control but remained same(5.55±0.53 ppm) which may be that fish felt some stress with aflatoxin treatment, became agitated and consumed comparatiovely more oxygen than normal which ultimately decreased overall level of dissolved oxygen in tank(Table 5).

Growth parameters: Initial weight taken was 2.51 gram in control group and 2.05 gram in all the other treatments. There was increase in weight but it remained very slow. Highest weight gain was observed in control group 6.11 gram was the maximum while it was the lowest in $T_5$where Aflatoxin was 50 ppb in the diet.There was gradual decrease in weight gain when we move from control to T_5 which showed significant impact of AflatoxinB_1 administration to fish. Weight increments were same in T_4 and T_5. Similarly it was same for T_3 and T_4. Among treated groups weight gain was the highest in $T_1$and T_2; it appeared that toxicity actually started from T_3 when concentration was 30ppb. Significant effect of AflatoxinB_1 could not be observed up to 20 ppb.

The effect of AflatoxinB_1on weight increment was less prominent at 10 ppb concentration. Final weight, net weight gain, percent weight gain, specific growth rate, followed the same trend because all these values are

dependent on actual weight by the fish at the end of the growth period. Specific growth rate (SGR) was very low in T_4 and T_5 significantly lower than all their counter parts. Survival rates however contradicted to weight gain to some extent. Survival remained 100% up to T_3 then declined and remained 80% in T_5 and 90% in T_4.

The values were significantly lower than all the remaining treatments as well as from the control. Feed conversion ratio (FCR) values were relatively very high when we compared them with the peak growth periods at ideal growth temperature. One possible reason can be that the weather was slightly cold, fish might took little feed than it should but drastic increase in feed conversion ratio (FCR) values in the latter treatments is strong evidence of effect of Aflatoxin B_1 on fish growth, FCR values, SGR etc. Whatever the weather was it is very clear that Aflatoxin B_1 present in fish feed drastically changed the metabolic and physical behaviour of fish (Table 4).

Body composition of fish: There were drastic changes in body composition. Protein gradually decreased from control to T5. It was significantly higher in control group when compared with its counter parts. The lowest values of protein were observed in T5 displayimg the magnitude of the effect ofaflatoxin. All the treated groups differed significantly from each other. It appeared that effects of aflatoxin mounted up with its rising concentrations. Similar trend was observed in ether extract. It was the highest in control and the lowest in T5. Moisture and ash contents followed the opposite. Where values of protein and ether extract decreased these parameters showed proportionate increase and effectively took their place (Table 6).

Histopathological studies results

Effects of Aflatoxin B_1 on kidney: In Fig1T5(5) There was vacuolation in tubular epithelial area with heavy infiltration of mononuclear cells and pyknotic nuclei of hepatocytes. Renal tubules are completely collapsed with their obliterated lumen

Fig2 T5(3) tubular disintegration with pyknotic nucleus & loss of cytoplasm seen very prominent. Free RBC's are present in interstitial areas indicate vascular haemorrhages.

Effects of Aflatoxin B_1 on Intestine: Fig3 T5(2) Sloughing of surface epithelial cells with Infiltration of mononuclear cells especially plasma cells in lamina propria is prominent

Effects of Aflatoxin B_1 on Liver: Fig 4 T5(3):- Granular cytoplasm of some hepatocytes indicative of cellular swelling and severe vacuolation.And peripheral elongated nucleus rather than normal central round. Fig 5 T4(3):- In hepatocytes cytoplasm moderate vacuolation is seen clearly.

Table 2. Average body weight of advance fry *Catla catla* in control and Aflatoxin B_1 treated aquariums

Fortnights	Control		T_1 (10 ppb)		T_2 (20 ppb)		T_3 (30 ppb)		T_4 (40 ppb)		T_5 (50 ppb)	
	Average body weight (g)	Weight gain	Average body weight (g)	Weight	Average body weight (g)	Weight gain	Average body weight (g)	Weight gain	Average body weight (g)	Weight gain	Average body weight (g)	Weight gain
15-09-2011 (Stocking)	2.51±0.04^{g}	-	2.40±0.12^{g}	-	2.10±0.01^{g}	-	2.27±0.13^{g}	-	2.14±0.07^{g}	-	2.05±0.16^{f}	-
30-09-2011	3.49±0.02^{f}	1.02±0.02^{ab}	3.26±0.01^{f}	0.90±0.02^{abc}	3.02±0.02^{f}	0.74±0.01^{b}	2.81±0.02^{f}	0.59±0.02^{b}	2.48±0.09^{f}	0.43±0.03^{b}	2.27±0.02^{e}	0.20±0.02^{b}
15-10-2011	4.54±0.03^{e}	1.04±0.02^{ab}	4.16±0.04^{e}	0.93±0.02^{ab}	3.80±0.73^{e}	0.73±0.02^{b}	3.30±0.14^{e}	0.55±0.03$_{bc}$	2.96±0.02^{e}	0.42±0.01^{b}	2.40±0.09^{e}	0.23±0.02^{b}
30-10-2011	5.61±0.01^{d}	1.04±0.02^{ab}	5.10±0.02^{d}	0.85±0.07^{bc}	4.52±0.01^{d}	0.75±0.01^{b}	3.95±0.02^{d}	0.52±0.02^{bc}	3.35±0.07^{d}	0.42±0.01^{b}	2.65±0.07^{d}	0.23±0.01^{b}
15-11-2011	6.62±0.02^{c}	1.01±0.01^{ab}	5.94±0.02^{c}	0.86±0.02^{bc}	5.23±0.03^{c}	0.72±0.01^{b}	4.43±0.02^{d}	0.48±0.03^{bc}	3.81±0.03^{c}	0.41±0.04^{b}	2.93±0.02^{c}	0.20±0.02^{b}
30-11-2011	7.63±0.07^{b}	1.04±0.01^{ab}	6.70±0.16^{b}	0.88±0.03^{bc}	5.98±0.01^{b}	0.72±0.01^{b}	4.86±0.14^{c}	0.49±0.02^{c}	4.30±0.02^{b}	0.43±0.02^{b}	3.16±0.02^{b}	0.23±0.01^{b}
15-12-1011	8.72±0.01^{a}	1.06±0.01^{a}	7.64±0.02^{a}	0.82±0.02^{c}	6.76±0.07^{a}	0.74±0.01^{b}	5.74±0.02^{a}	0.52±0.01^{c}	4.73±0.02^{a}	0.46±0.01^{b}	3.36±0.02^{a}	0.23±0.01^{b}

Note: Data figures with different superscript letters are significantly different from each other at P<0.05

Table 3. Average body length of advance fry of *Catla catla* in control and Aflatoxin B_1 treated aquariums

Fortnights	Control		T_1 (10 ppb)		T_2 (20 ppb)		T_3 (30 ppb)		T_4 (40 ppb)		T_5 (50 ppb)	
	Average body length (cm)	Length gain (cm)	Average body length (cm)	Length gain (cm)	Average body length (cm)	Length gain (cm)	Average body length (cm)	Length gain (cm)	Average body length (cm)	Length gain (cm)	Average body length (cm)	Length gain (cm)
15-09-2011 (Stocking)	5.81±0.01^{g}	-	5.72±0.01^{g}	-	5.81±0.01^{d}	-	6.03±0.09^{a}	-	5.83±0.02g	-	5.72±0.01^{g}	-
30-09-2011	6.86±0.01^{f}	1.07±0.02^{ab}	6.56±0.04^{f}	0.82±0.02^{a}	6.73±0.02^{d}	0.74±0.01e	6.48±0.01^{a}	0.53±0.01^{dc}	6.31±0.01^{f}	0.44±0.01^{bc}	6.04±0.07^{f}	0.21±0.01^{c}
15-10-2011	7.97±0.02^{e}	1.13±0.01^{a}	7.43±0.02^{e}	0.86±0.01^{a}	6.52±1.42^{d}	0.78±0.00b	7.04±0.01^{a}	0.54±0.01^{bc}	6.74±0.02^{e}	0.43±0.01^{b}	6.21±0.01^{e}	0.23±0.01^{b}
30-10-2011	9.06±0.01^{d}	1.07±0.01^{ab}	8.26±0.02^{d}	0.82±0.01^{a}	8.29±0.02^{c}	0.78±0.^{01a}	7.79±0.28^{a}	0.56±00^{a}	7.15±0.07^{d}	0.44±0.07^{b}	6.46±0.02^{d}	0.22±0.01^{bc}
15-11-2011	10.11±0.01^{c}	1.04±0.01^{b}	9.12±0.01^{c}	0.82±0.01^{a}	9.03±0.02^{bc}	0.72±0.01^{a}	8.16±0.01^{a}	0.55±0.01^{ab}	7.63±0.02^{c}	0.44±0.01^{b}	6.65±0.02^{c}	0.22±0.01^{bc}
30-11-2011	11.15±0.03^{b}	1.06±0.01^{ab}	9.94±0.02^{b}	0.85±0.01^{a}	9.74±0.01^{ab}	0.68±0.01^{c}	8.64±0.04^{a}	0.51±0.00^{e}	8.06±0.01^{b}	0.41±0.00^{c}	6.93±0.02^{b}	0.23±0.00^{b}
15-12-1011	12.25±0.01^{a}	1.05±0.01^{ab}	10.83±0.02^{a}	0.83±0.03^{a}	10.40±0.02^{a}	0.64±0.01^{d}	4.84±6.13^{a}	0.52±0.01^{de}	8.47±0.02^{a}	0.42±0.01^{bc}	7.16±0.02^{a}	0.23±0.01^{b}

Note: Data figures with different superscript letters are significantly different from each other at P<0.05

Table 4. Growth parameters of advance fry *Catlacatla* under control and Aflatoxin B_1 treated aquariums

Parameters	Control	T_1 (10 ppb)	T_2 (20 ppb)	T_3 (30 ppb)	T_4 (40 ppb)	T_5 (50 ppb)
No. of fish stocked	15	15	15	15	15	15
Initial Weight (g)	2.51±0.354^a	2.40±0.141^a	2.30±0.282^a	2.27±0.162^a	2.24±0.148^a	2.05±0.177^a
Final Weight (g)	8.86±0.198^a	7.89±0.254^b	6.88±0.169^c	5.87±0.184^d	4.86±0.190^e	3.68±0.452^f
Net weight gain (g)	6.11±0.148^a	5.32±0.113^b	4.58±0.113^c	3.48±0.021^d	2.62±0.042^e	1.50±0.275^f
Percent weight gain	223.71±33.524^a	213.01±7.521^a	200.95±9.627^a	146.43±9.093^a	116.89±8.843^a	68.90±7.080^a
Specific Growth Rate (SGR %)	3.40±0.282^a	3.14±0.169^a	3.20±0.247^a	2.60±0.106^b	2.19±0.919^b	1.51±0.120^c
Feed Conversion Ratio (FCR)	6.14±0.194^e	8.52±0.180^c	7.10±0.141^d	11.03±0.142^b	11.28±0.395^b	17.46±0.657^a
Survival rate (%)	100^a	100^a	100^a	100^b	90^b	80^b

Table 5.Physico-chemical analysis of control and treated with Aflatoxin B_1 in aquariums

Parameters	Control	T_1 (10 ppb)	T_2 (20 ppb)	T_3 (30 ppb)	T_4 (40 ppb)	T_5 (50 ppb)
Water Temprature^{0}C	45.72±0.000^a	45.60±0.148^a	45.60±0.000^a	45.53±0.233^a	45.48±0.155^a	45.47±0.042^a
Dissolved Oxygen(DO)	5.57±0.042^a	5.54±0.148^a	5.51±0.141^a	5.52±0.374^a	5.47±0.014^a	5.26±0.106^a
pH	7.59±0.007^{bc}	7.85±0.056^a	7.52±0.028^c	7.56±0.056^{bc}	7.61±0.021^{bc}	7.66±0.049^b
Alkalinity	2.47±0.035^b	2.59±0.063^a	2.49±0.014^b	2.57±0.035^a	2.49±0.007^b	2.58±0.028^a
(TSS)	0.22±0.000^a	0.23±0.000^a	0.28±0.000^a	0.18±0.021^a	0.25±0.070^a	0.25±0.070^a
Hardness	11.27±0.000^b	11.55±0.00^{ab}	11.65±0.212^a	11.42±0.106^{ab}	11.42±0.106^{ab}	11.56±0.056^a
Nitrate	0.15±0.035^a	0.13±0.014^a	0.13±0.049^a	0.11±0,028^a	0.14±0.007^a	0.15±0.035^a
Ammonia	0.175±0.021^c	0.140±0.028^d	0.165±0.141^b	0.130±0.007^b	0.145±0.035^a	0.175±0.007^b
Chloride	24.38±0.162^b	24.28±0.728^b	24.45±0.070^{ab}	24.35±0.070^b	25.28±0.304^a	24.56±0.049^{ab}

Table 6. Proximate Analysis of advance fry*Catla catla*in control and treated with Aflatoxin B_1 in aquaria

	Control	T_1 (10 ppb)	T_2 (20 ppb)	T_3 (30 ppb)	T_4 (40 ppb)	T_5 (50 ppb)
Crude Protein (%)	56.3± 0.14^a	54.3±0.14^b	51.3 ±0.14^c	49.3±0.07^d	44.9 ±0.28^e	43.5 ±0.14^f
Crude Fat (%)	23.3± 0.14^a	21.5±0.07^b	20.2 ±0.21^c	17.1 ±0.28^d	14.8±0.21^e	13.4±0.14^f
Moisture (%)	75.2±0.14 ^{e}	76.2 ±0.07^d	78.2 ±0.21^c	82.4± 0.77^b	83.8 ±0.14^a	84.3 ±0.21^a
Ash (%)	20.2 ±0.21^f	23.2± 0.28^e	24.6±0.14^d	28.2 ±0.21^c	32.4 ±0.07^b	35.6 ±0.00^a

Advance fry Kidney

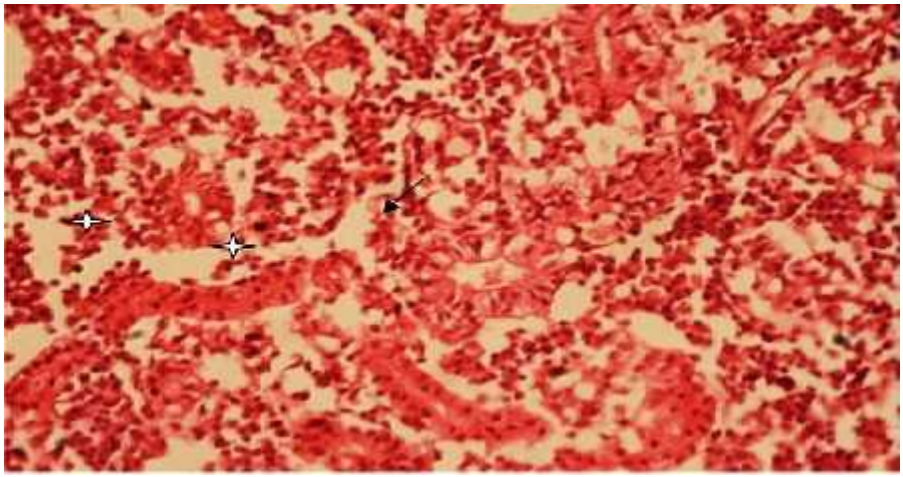

Fig (1)T5(5):- Note heavy Infiltration of mononuclear cells. Vacuolation in tubular epithelial cells seen with pyknotic nuclei (Arrow). Renal tubules are completely collapsed with their obliterated lumen (star). H&E; x40

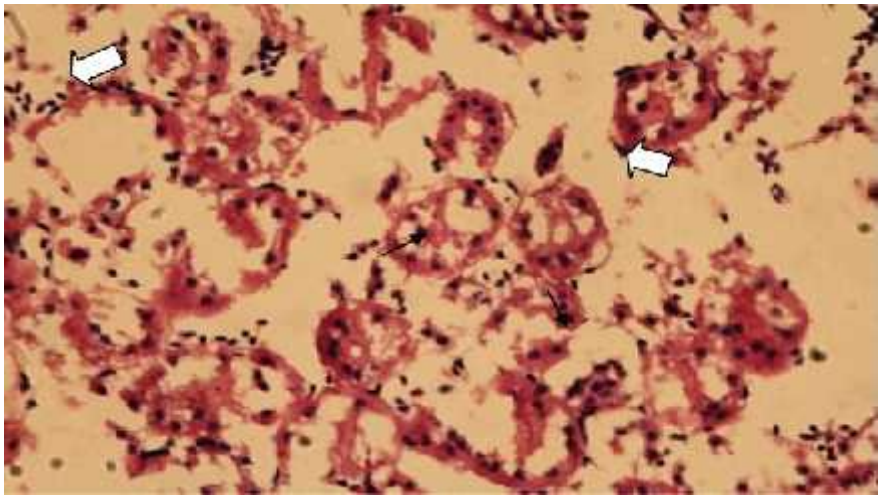

Fig (2)T5(3):- Tubular disintegration seen very prominent with pyknotic nucleus & loss of cytoplasm (Black arrow). Free RBC's are present in interstitial areas indicate vascular haemorrhage (White arrow). H&E; x40

Advance fry Intestine

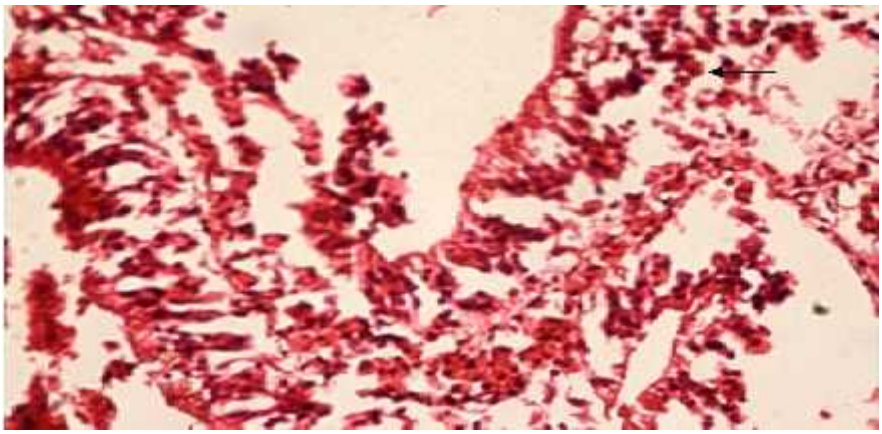

Fig (3)T5(2):-Infiltration of mononuclear cells (Arrow) especially plasma cells in lamina propria. Sloughing of surface epithelial cells. H&E; x40

Advance fry Liver

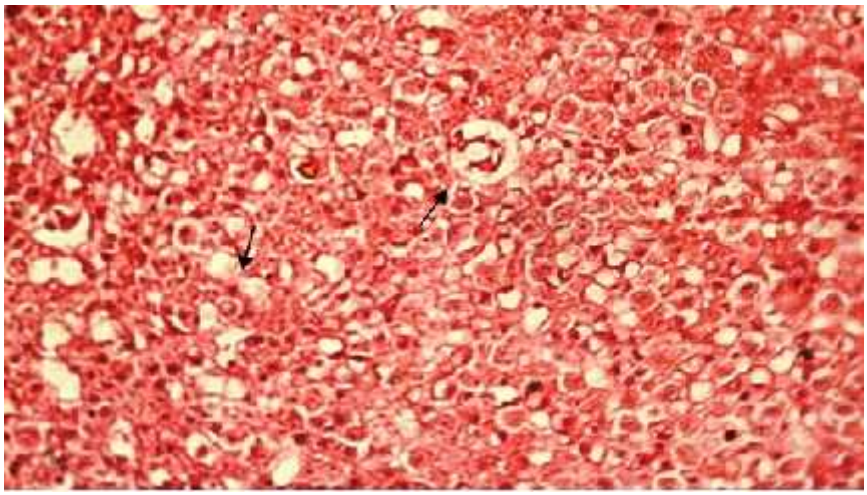

Fig (4) T5(3):- Note severe vacuolation with peripheral elongated nucleus rather than normal central round (Black arrow). Granular cytoplasm of some hepatocytes indicative of cellular swelling. H&E ; x40.

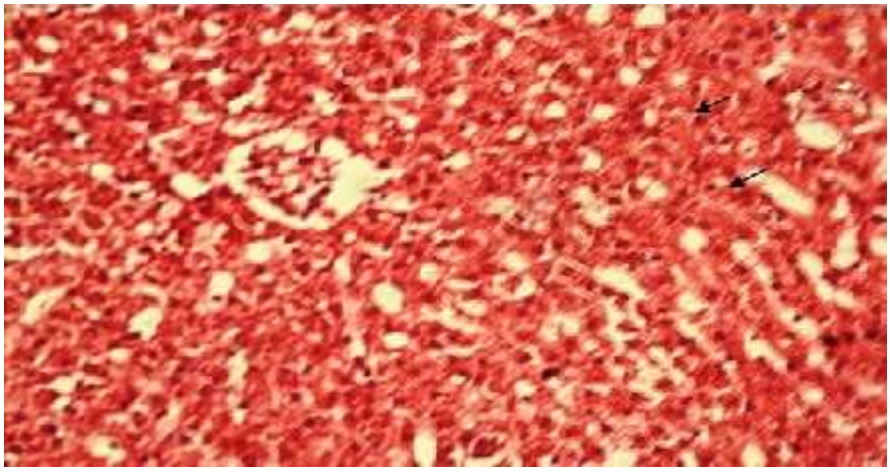

Fig (5)T4(3):- Moderate vacuolation in hepatocytes cytoplasm (Arrow). H&E; x40

DISCUSSION

The body weight of advance fry *Catlacatla* was the highest in control group as compared to aflatoxins treated aquariums. Results revealed that growth of aflatoxicosed fish was significantly different and lower than control ($p< 0.05$). Han *et al*., (2010) who observed the effects of Aflatoxin B_1 (AFB_1) on growth, physiological responses and histological changes were investigated in juvenile gibel carp (*Carassiusauratusgibelio*). The results were contradicted with fish weight gain fed Diet 6 was 112.6% of that of control group (Diet 1) after 3 months, but there was no significant difference of weight gain between fish fed Diet 7 and the control group.

Specific growth rate was higher in control aquarium, lowest was in T5 where 50 ppb aflatoxins were applied. Feed conversion ratio was highest in T5 as compared to control. The survival rate was 100% upto T3 while in T4 and T5 was lowest. Sepahdar*et al*.,(2009) in their experiment observed the various levels of AFB1 which do not significantly affect the specific growth ratio (SGR) ($P<0.05$) of fish in different treatments. Weight gain and food conversion ratio (FCR) varied significantly ($P<0.05$) between control and treatments with diets contaminated with 10 ppb to 50 ppb (AFB1/kg after 90 days). He suggested that various AFB1 levels under experimental conditions of the present study affect some growth factors, such as, weight gain and FCR and histopathological studies showed that different level of AFB1 can cause change in liver tissue, including progressive fat deposition, hepatocyte degeneration and necrosis, particularly at concentration of 75 and 100 ppb AFB1/kg of diets after 60 days.

A general reduction in feed intake in fish fed with high AFB1 levels which has been earlier documented (Jantrarotai*et al*., 1990; Cha` vez-Sa` nchez*et al*., 1994) was observed in the present study also, especially in T1 (10ppb) to T5 (50 ppb) with higher level of Aflatoxin B_1.

Although the feeding rate was gradually increased according to their body weight as a result of corresponding decline in weight gain and SGR could not be seen in these treatments.This feeding rate was observed among fish from groups T1 to T5 as a result of the abrupt decline of feeding intake during the last fortnights of the trial when a mortality was observed in T4 anf T5. Same trends was observed by Rajeev *et al*. 2011 during his study.

Dietary toxins are sometimes known to have stimulant properties. Dietary microcystin has been known to increase feeding rate in Nile tilapia, *O. niloticus* (Zhao *et al*. 2006a) gibel carp, *Carrasius auratus gibelio* (Zhao *et al*. 2006b) as well as hybrid sturgeon, (Zhao 2006). In a similar experiment from our laboratory (Han *et al*., unpublished data), dietary AFB_1 was also observed to possess stimulant properties leading to an increased feeding rate in juvenile gibel carp. These observations show that the impact of various dietary toxins on feeding rate is largely species specific. Hendricks *et al*., (1980) suggested that negative effects of AFB_1 on growth could be observed only after longer periods of feeding with the toxin which could be as long as 16 months in trout.In another study, no significant reduction in weight gain could be observed in channel catfish when fed experimental diets with AFB_1 levels ranging from 100 to 2154 pg kg^{-1}) (Jantrarotai& Lovell 1990). Manning *et al*., (2005) opined that aflatoxin contamination from moldy corn caused no reduction in channel catfish performance. Similarly, growth reductions in tilapia were seen only after they were fed a diet with AFB_1 levels as high as 10 mg kg^{-1} (Tuan *et al*., 2002). The fact that no growth reductions were seen when fish were fed higher levels of AFB_1(100–2154 pg kg^{-1}) (Jantrarotai& Lovell 1990; Tuan *et al*., 2002) than those used in the present study (5–80 pg kg^{-1}) provide strength to our observations on the insignificant differences in growth between groups.

Han *et al*.,(2010) obsrved that fish fed with more than 100 AFB_1 kg^{-1} diet showed impaired physiological

responses and more AFB_1 residue of muscles and ovaries above the safety limitation of European Union which occurs naturally in several important plant feedstuffs.

Collapsed liver were found in dead fish in T4 and T5. Similar manifestations were reported by Roberts (1978), Ferguson (1989), and Royes *et al.*, (2002).Several histological alterations were recognized in the liver of the fish examined and these were chronic manifestations.The liver of fishes in T2, T3 and T4 had extensive necrosis, acute cellular swelling or ballooning necrosis, chronic granulomatous inflammation, loss of color where the necrotic tissue becomes paler than the surrounding normal tissue. Joner (2000) described the effect of aflatoxin in the liver as follows: first, aflatoxin is absorbed from the diet in the alimentary canal and is passed to different organs. The principal target organ for aflatoxins is the liver. After the invasion of aflatoxins into the liver, lipids infiltrate hepatocytes and leads to necrosis or liver cell death. The main reason for this is that Aflatoxin metabolites react negatively with different cell proteins, which leads to inhibition of carbohydrate and lipid metabolism and protein synthesis. In relation with the decrease in liver function, there is a dearrangement of the blood clotting mechanism, jaundice, and a decrease in essential serum proteins synthesized by the liver.

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