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Aflatoxin Effect of Moulded Gala Waste Mixed with Ginger and its Histopathological Study on *Clarias Gariepinus*

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I. Introduction

Plant-based ingredients are increasingly used in fish diets due to increase in economic/market pressures on feed compounders to produce lower cost and sustainable alternatives. The increased reliance on commercially prepared feed formulated with higher levels of grain material means that fish have the same risk of potential exposure to mycotoxins as terrestrial agricultural species.

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. Mycotoxins are secondary metabolites produced by certain filamentous fungi, which can be produced in foods as a result of fungal growth. They cause a toxic response, termed a mycotoxicosis, when ingested by higher vertebrates and other animals. Consumption of mycotoxin contaminated foods has been associated with several cases of human poisoning, or mycotoxicosis, sometimes resulting in death (Sweeney and Dobson, 1998 and Bathnagar and Garcia, 2001). Aflatoxins are considered the most carcinogenic, mutagenic and teratogenic poisonous by-products of the growth of the moulds *Aspergillus flavus* and *Aspergillus parasiticus*, and are important contaminants of certain foods and animal feeds because of their ability to produce aflatoxins (Farr *et al.*, 1989).

Aflatoxin losses to livestock and poultry producers from aflatoxin-contaminated feeds include death and more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency (Vincelli *et al.*, 1995 and FAO 1997 & 2002).

Therefore, some scientific efforts were conducted to use the herbs or natural plants which, detoxifies the drastic effects of mycotoxins or aflatoxins on some animals such as, glucomannan (Karaman *et al.*, 2005) or yeast cell wall mannanoligosaccharide (MOS) (Devegowda *et al.*, 1998), or Saccharomyces cerevisiae which were found to have beneficial effects in poultry during mycotoxicosis (Raju and Devegowda 2000), chamomile (Abdelhamid *et al.*, 1985; Soliman and Badeaa 2002 and Ibrahim, 2004), ginger (Vimala *et al.*, 1999 and Abdelhamid *et al.*, 2002c).

The expansion of global aquaculture is increasing the demand for aquaculture feed which is the prime input in fish culture practices. Generally, the selection of feed ingredients for any Production system depends upon its nutritional value 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 at an early phase.

Fish meal is used globally at a dietary protein in formulated fish seeds but the major problems with the

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use of fish meal as source of protein in the fish diet are its rising cost, uncertain availability, adulteration and variation in quality (Prabjeet *et al*, 2011). The increasing demand, unstable supply and high price of the fish meal with the expansion of aquaculture made it necessary for search for alternative protein source (FAO 2004; Lunger *et al*. 2007). Hence there is need to replace this ingredient partially or fully with some other suitable ingredients to reduce the production cost (Prabjeet *et al*, 2011).

The study therefore aimed at examining the additive nature of ginger on inclusion level of the waste sausage (Gala) waste and to determine the histopathological effect of the experimental feed on the fish visceral organs.

II. Materials and Methods

One hundred and eighty experimental fingerlings of *Clarias gariepinus* with average weight of 3.9±0.02 were purchased from a private farm and randomly stocked into twelve (1.0m x 1.0m x 1.0m) happas of 15 fingerlings each. The fish were allowed to acclimatize to the environment for a period of two weeks before the experiment began, during the two weeks period all the fish were fed two times daily with commercial feed (coppen 2.0mm).

The experiment was carried out in 12m x12m x 1.5m earthen pond, replicated thrice. The hapas were suspended and stabilized in the water body using kuralon twine tied horizontally into the bottom substratum.

Table 1

1	2	3	4	5	6	7	8	9	10	11	12
T2R2	T3R3	T3R2	T1R3	T2R1	T4R2	T1R2	T3R1	T4R3	T1R1	T2R3	T4R1

Treatment 1- Control Diets (0% inclusions)

Treatment 2- Ginger (10% inclusion)

Treatment 3- Ginger (20% inclusion)

Treatment 4- Ginger (30% inclusion)

III. Experimental Procedure

A total of 180 fingerlings of *C. gariepinus* were stocked in the net happa. The fingerlings were selected randomly, Fifteen fingerlings were selected into each net hapa, weighed with a sensitive electronic weighing scale (meter toledo FB602) and fed at 3% body weight twice daily for a period of 8weeks between the hours of 07:00-08:00 and 16:00-17:00. Sampling was carried out bi-weekly and the amount of feed was adjusted accordingly with increased in their body weight, mortality was also monitored daily with their response to feed.

a) Growth Performance

The following growth performances were measured:

$$\text{Percentage weight gain PWG (\%)} = \frac{(\text{Final mean body weight})}{(\text{Initial mean body weight})} \times 100$$

$$\text{Specific growth rate, SGR} = \frac{L_n W_2 - L_n W_1 \times 100}{\text{Time (days)}}$$

W1= initial weight gained

W2= Final weight gained

Ln= Natural logarithm

$$\text{Protein efficiency ratio} = \frac{\text{Mean weight gain}}{\text{Average protein fed}}$$

$$\text{Feed conversion ratio} = \frac{\text{weight of feed (g)}}{\text{Weight gained}}$$

$$\text{Mortality rate} = \frac{\text{No of fish dead at the end of the experiment} \times 100}{\text{No of fish at the beginning of the experiment}}$$

$$\text{Survival rate} = \frac{\text{No of fish remaining at the end of the experiment} \times 100}{\text{No of fish at the beginning of the experiment}}$$

Feed conversion ratio, FCR is obtained by dividing the total weight of the food administered to the total increase in weight gained by the fish over a period of time.

$$SGR = \frac{L_n W_2 - LW_1 X100}{\text{Time (days)}}$$

W_1 = Initial weight gain

W_2 = Final weight gain

L_n = Natural logarithm

Time = Number of days of experiment

$$PER = \frac{\text{Fish weight gain}}{\text{Protein gain}}$$

Table 2 : Feed Ingredient.

Ingredients	T1 Control diet	T2	T3	T4
Maize	17.4	17.4	17.4	17.4
Fish meal	34.94	34.94	34.94	34.94
Soybean meal	17.47	17.47	17.47	17.47
Groundnut cake	17.47	17.47	17.47	17.`47
Gala	8.70	8.70	8.70	8.70
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Vit. Premix	1	1	1	1
Ginger	-	0.2(10%)	0.4(20%)	0.6(30%)
Salt	0.5	0.5	0.5	0.5
Total	100	100	100	100

Table 3 : Proximate analysis of the experimental diet.

Parameters	T1	T2	T3	T4
Moisture	7.6 ± 0.01^{c}	9.73 ± 0.01^{a}	9.15 ± 0.01^{b}	6 ± 0.06^{d}
Dry	92.98 ± 0.10^{b}	90.27 ± 0.23^{d}	90.85 ± 0.10^{c}	94 ± 0.10^{a}
Fat	19.57 ± 0.05^{a}	13.54 ± 0.04^{d}	16.52 ± 0.01^{b}	15.02 ± 0.01^{c}
Ash	9.26 ± 0.09^{a}	7.96 ± 0.11^{b}	8.23 ± 0.08^{b}	8.15 ± 0.02^{b}
F.C	3.56 ± 0.03^{a}	2.17 ± 0.01^{d}	2.31 ± 0.05^{c}	2.41 ± 0.00^{b}
C.P	31.68 ± 0.05^{d}	38.68 ± 0.20^{c}	39.24 ± 0.06^{b}	41.23 ± 0.06^{a}
CHO	28.91 ± 0.01^{a}	27.95 ± 0.03^{b}	24.48 ± 0.01^{b}	26.54 ± 0.01^{c}

Means along the same row with different superscripts are significantly different ($p<0.05$).

IV. Statistical Analysis

All data obtained were subjected to one-way ANOVA test Where ANOVA revealed significant differences ($P<0.05$), Duncan's multiple-range test (Zar, 1996) was applied to characterize and quantify the differences between treatments using SAS software for windows (SAS, 2009).

V. Results and Discussion

Table 4 : Carcass Analysis of experimental fish.

Parameters	Initial	T1	T2	T3	T4
Moisture	78.62±0.30[b]	78.47±0.03[b]	77.52±0.22[c]	78.81±0.23[b]	80.67±0.06[a]
Dry	21.38±0.01[b]	21.53±0.06[b]	22.48±0.23[a]	21.19±0.03[b]	19.33±0.02[c]
Fat	2.79±0.05[c]	2.81±0.01[c]	4.50±0.11[a]	4.04±0.01[b]	2.93±0.15[c]
Ash	1.44±0.01	6.72±5.04	2.04±0.01	1.96±0.03	1.71±0.05
F.C	0.98±0.21	1.06±0.03	1.45±0.24	1.37±0.02	1.09±0.03
C.P	37.84±0.17[d]	43.23±0.03[a]	41.57±0.01[b]	40.60±0.12[c]	40.44±0.12[c]
CHO	1.22±0.05[c]	1.52±0.04[a]	1.37±0.01[b]	1.18±0.05[c]	0.98±0.02[d]

Means along the same row with different superscripts are significantly different ($p<0.05$).

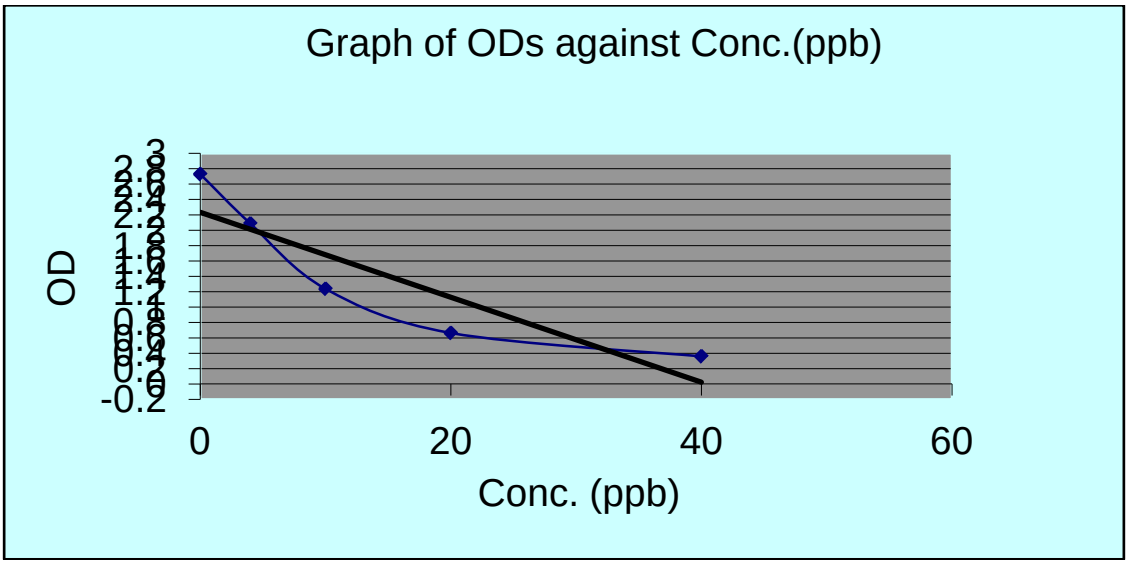
The initial and final carcass analysis of the fish fed with different experimental diet is represented in Table 4. The final moisture content of the fish was high in two Treatments, Treatment 4 (80.67±0.06) which was followed by treatment 3 (78.81±0.23).

Compared to the initial values while treatment 2 was low with treatment 4 having the highest moisture content (80.67±0.06) which was followed by treatment 3 (78.81±0.23).

However, T1 recorded the highest crude protein (43.23) and the lowest was recorded for T4 (40.44). Crude fibre ranged between 1.06 in T2 to 1.45 in T1, Carbohydrate ranged from 40.44 to 43.23. Fat was highest in Treatment 2 followed by Treatment 3.

Table 5 : The Result of Total Aflatoxin Level in Moulded Gala.

X-axis	Y-axis
0	2.034
4	1.741
10	0.990
20	0.644
30	0.350



By extrapolation from the standard curve and multiplication by dilution factor (10), value of total aflatoxin in sample is 69.7 ppb (µg/kg).

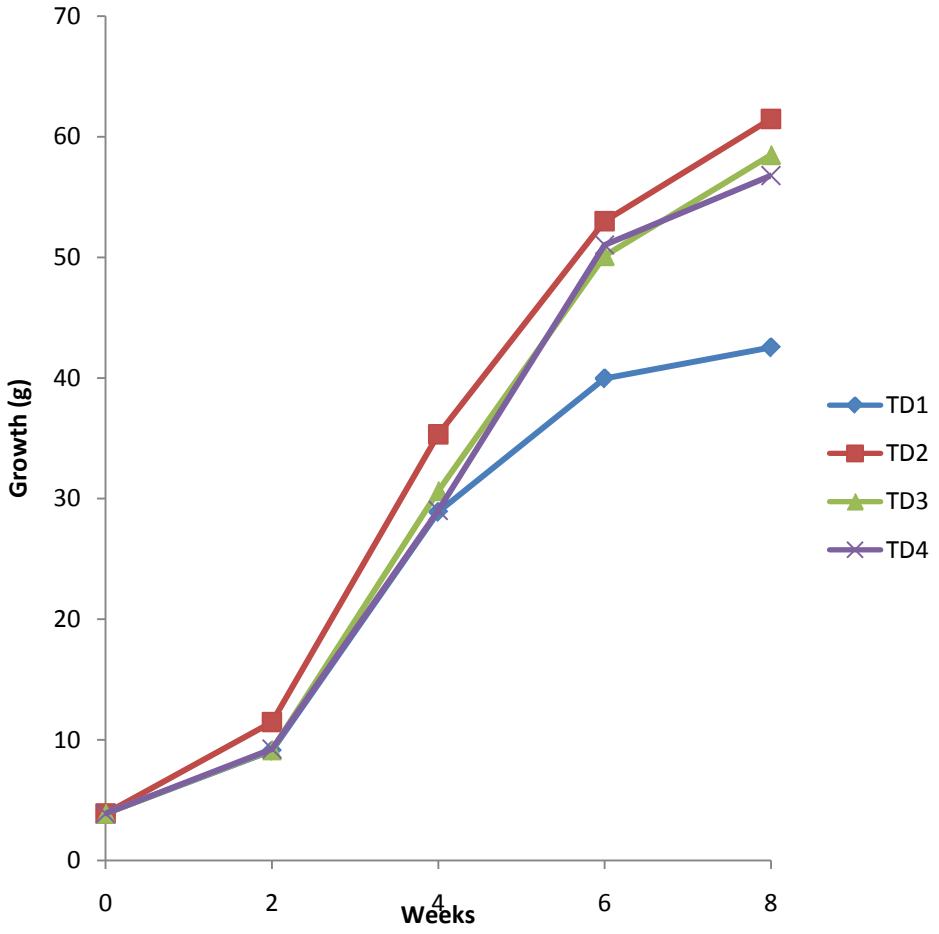
Table 6 : Growth response of fish to different percentage inclusion of Gala-Ginger in the feed.

Parameters	0% Inclusion	10%Inclusion	20%Inclusion	30%Inclusion
Initial Weight (g/fish)	3.90±0.02	3.90±0.04	3.90±0.03	3.90±0.02
Final Weight (g/fish)	40.00±2.89^{a}	110.33±5.17^{ab}	82.67±4.81^{b}	80.00±11.37^{b}
Weight Gain (g/fish)	36.10±2.89^{a}	106.43±5.17^{ab}	78.77±4.81^{b}	76.10±11.37^{b}
AFC(g/fish/day)	111.35±3.73^{a}	110.37±65.71^{a}	112.90±10.12^{a}	119.92±27.63^{b}
FCR	3.14±0.35^{a}	1.01±0.58^{b}	2.32±0.03^{a}	2.61±0.09^{a}
SGR	2.30±0.06^{b}	3.33±0.07^{a}	2.63±0.09^{b}	2.53±0.27^{b}
TPC	31.94±2.43^{b}	42.66±25.40^{b}	44.30±3.97^{a}	49.44±11.39^{a}
PER	0.87±0.10^{a}	1.03±0.06^{a}	0.91±0.01^{a}	0.39±0.22^{b}
SURVIVAL	40.00±10.18^{b}	68.89±2.22^{a}	44.44±15.55^{b}	48.89±8.01^{ab}

Means along the same row with different superscripts are significantly different ($p<0.05$).

AFC: Average Feed Consumed, FCR: Feed Conversion Ratio, SGR: Specific Growth Rate, TPC: Total Protein Consumed, PER: Protein Efficiency Ratio.

Figure 1 : Growth response of *Clarias gariepinus* fingerlings fed with ginger based diets.



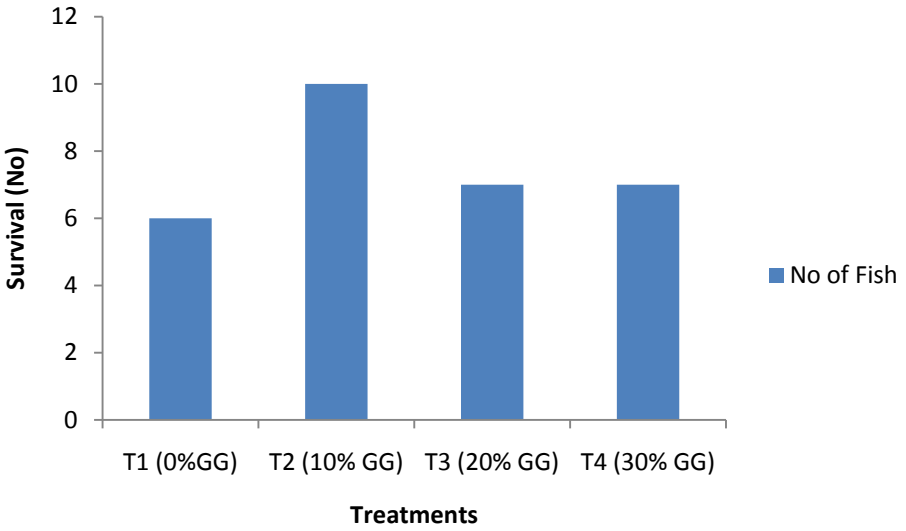


Table 7 : Physcio-chemical Parameters.

Weeks	pH	Dissolve oxygen (Mg/L)	Temp (°C)
0	7.20±0.06	6.17±0.03	26.00±1.15
1	7.31±0.12	6.30±0.20	26.53±0.27
2	7.45±0.32	6.23±0.33	27.17±0.33
3	7.41±0.11	6.53±0.13	27.37±0.07
4	7.25±0.43	7.40±0.80	26.40±0.35
5	7.15±0.20	7.47±0.73	28.01±0.39
6	7.55±0.55	7.25±0.14	27.71±0.19
7	7.26±0.20	7.33±0.33	27.44±1.12
8	7.80±0.30	7.40±0.20	26.78±0.77

Mean weekly values of physico-chemical parameters during the experimental period. Water quality parameters in the pond during the experimental period are represented in Table 3. The pH was between 7.15 -7.80, dissolve oxygen ranged between 6.17-7.47mgL-1 and temperature 26.00-28.01°C.

HISTOPATHOLOGY (NORMAL)

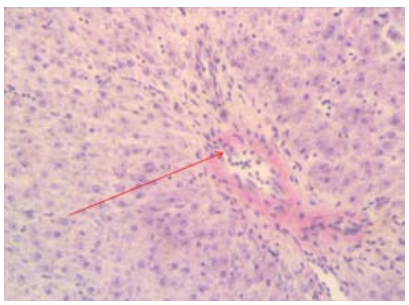


Plate 7
LIVER (TD1)
(Moderate diffuse Vascular Hepatic inoculation of hepatocytes

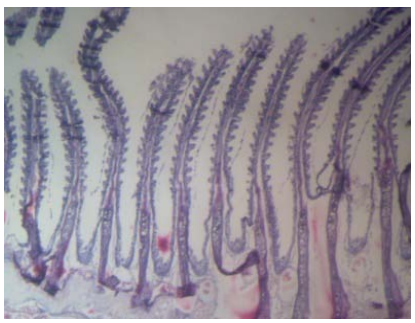


Plate 8
GILLS (TD1)
No visible lesion

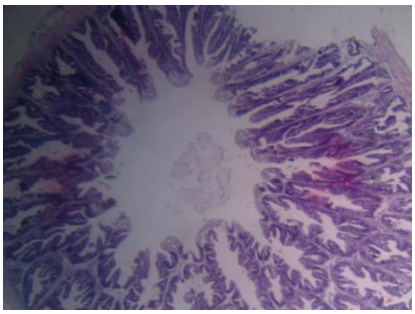
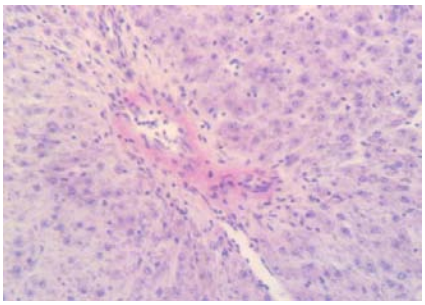
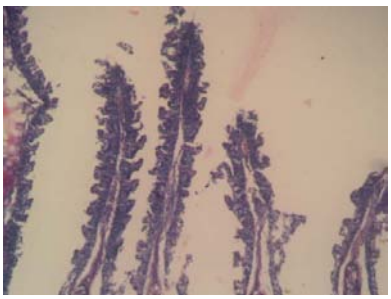


Plate 9
GUT (TD1)
No visible lesion

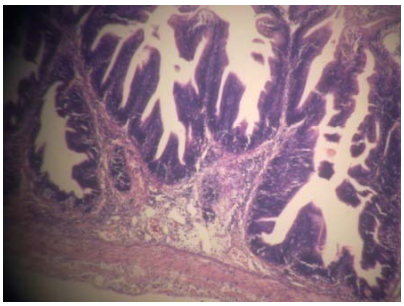
HISTOPATHOLOGY (TREATMENT 2)



Liver
Plate 10 (TD2)
Mild diffuse hepatic vacuolar Degeneration



Gill
Plate 11 (TD2)
No visible lesion



Gut
Plate 12 (TD2)
No visible lesion

HISTOPATHOLOGY (TREATMENT 3)

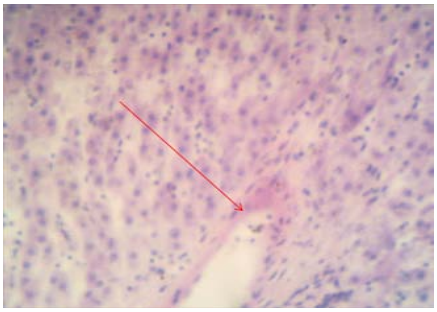


Plate 13
Liver (TD3)
Mild diffuse hepatic vacuolar degeneration

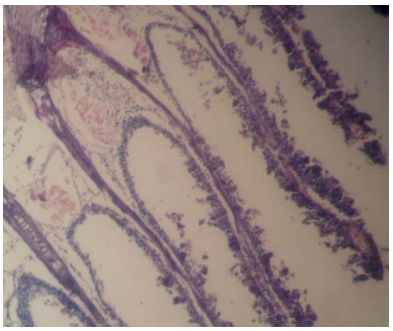


Plate 14
Gills (TD3)
No visible lesion

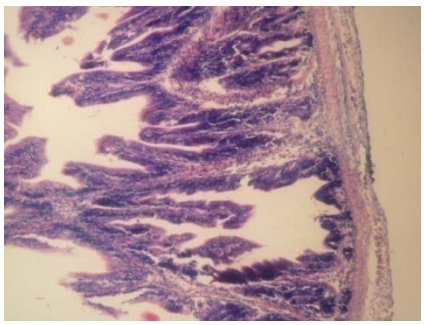


Plate15
Gut (TD3)
No visible lesion

HISTOPATHOLOGY (TREATMENT 4)

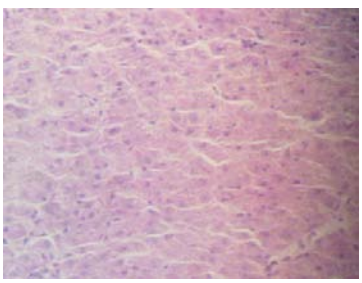


Plate 16
Liver (TD4)
No visible lesion (No physical damage)

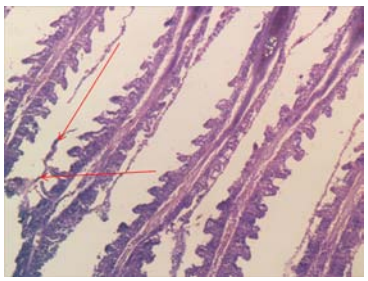


Plate 17
Gill (TD4)
Showing diffuse mild proliferation of the epithelial cells of the secondary lamellae

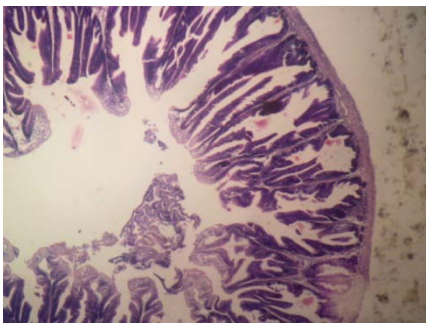


Plate 18
Gut (TD4)
No visible lesion (No physical damage)

VI. Discussion

There was a significant effects (Gala-Ginger inclusion) on final weight, Weight gain, FCR, SGR and PER ($p<0.05$), Protein efficiency ratio (PER) recorded the highest value in Treatment 2 (1.03 ± 0.06) and the lowest was recorded in Treatment 4 (0.39 ± 0.22). Feed conversion ratio (FCR), the highest value was recorded in Treatment 1 ($3.14\ \pm0.35$) and the lowest was recorded in Treatment 2 (1.01 ± 0.58). There was no significant difference ($p>0.05$) between Treatment 1 (3.14 ± 0.35), Treatment 3 (2.32 ± 0.03) and Treatment 4 (2.61 ± 0.09). Treatment 2 is the most effective (1.01 ± 0.58), it converts the feed to growth more than the other treatments.

Treatment 2 had the highest specific growth rate (3.33 ± 0.07) and the lowest value (2.30 ± 0.06) was recorded in Treatment 1.There was no significant difference ($p>0.05$) between the value obtained for Treatment 1 (2.30 ± 0.06), Treatment 3 (2.63 ± 0.09) and Treatment 4 (2.53 ± 0.27).

There was a significant difference ($p<0.05$) between mean weight gain in the value recorded for Treatment 1 (36.10 ± 2.89), Treatment 3 (78.77 ± 4.81) and Treatment 4 (76.10 ± 11.37). A similar result was recorded in final weight gain which complement the results obtain in the FCR (feed conversion ratio), SGR (Specific growth rate) and survival. This is similar to Ceylan *et al.* (1998) who reported that antibiotic supplementation to rye-based diets had no significant effect on the feed conversion ratio of broilers. Similar results were reported by Garcia *et al.* (1999), Aksoy *et al.* (1995) and Jamroz *et al.* (1995) who tested the effect of a xylanase enzyme preparation and Vukic-Vranjes and Wenk (1993) who compared the supplementation of an antibiotic supplement alone or combined with an enzyme. From this, 10% inclusion level is more efficient as recorded in FCR, SGR, final weight gain and survival rate.

The physico- chemical parameters of water were within the range for culturing African catfish, C *gariepinus* (Vivien *et al*,. 1977, Adekoya *et al*., 2004 and Omotayo *et al* 2006) recommended that dissolve oxygen (DO) values observed during the experimental period was 8mg/ litre in the pond and DO values observed during the experimental period fall within these values. The values of physico- chemical parameters observed in the pond were within the range recommended for *C. gariepinus* (Adekoya *et al*., 2004).

The histopathological examination of the liver in the experimental fish in Plate 7(control) shows moderate spread of disease on the liver cells. This is similar to the findings of (Abbas and Ali, 2007) who observed destruction and vacuolation of the muscle cells in *Oreochromis spp* exposed to chromium. Plate10 (Treatment 2), and Plate 13(Treatment 3) indicate deterioration of the membrane containing fluid in the cytoplasm of the liver cells. The vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchyma cells and the rate of their release into the circulation system (Gingerich, 1982). Plate 16 (Treatment 4) shows no physical change in the liver cells.

The histopathological examination of the gills in the experimental fish in Plate 8(Treatment 1), (Treatment 2) and (Treatment 3) in plate 8, 11 and 14 shows no physical change in the gills.The gills, participate in many important functions in the fish, such as respiration, osmoregulation and excretion. (Treatment 4) plate 17 shows slow diffuse mild proliferation of the epithelial cells of the secondary lamellae i.e. partial fusion of the secondary lamellae. This is similar to the findings of Ayotunde *et al.* (2011) who worked on Histological Changes in *Oreochromis niloticus* exposed to Aqueous extract of *Moringa oleifera* Seeds Powder .The cellular damage observed in treatment 4 in the gills in terms of epithelial proliferation, separation of the epithelial layer from supportive tissues and necrosis can adversely affect the gas exchange and ionic regulation (Dutta *et al*., 1993) and epithelial lifting are defense mechanisms. The present results are in agreement with those observed in other fish species under the influence of different pollutants (Olurin *et al*., 2003) (Camargo and

Martinez, 2007). In this respect, Camargo (2007) observed hyperplasia of the epithelial cells, fusion of secondary lamellae, lifting of the lamellar epithelium and blood congestion in the gills of *P. lineatus* caged in Cambé stream, Brazil, polluted by industrial, domestic and agricultural wastes. Also, (Triebskorn,*et al*., 2008) noticed epithelial lifting, proliferation of epithelial cells of primary and secondary lamellae, hyperplasia of mucous cells and necrosis of epithelial cells in the gills of *C. nasus* and *L. cephalus* from River Mures, Western Romania, polluted by heavy metals, faecal coliforms and streptococci bacteria.

The histopathology of the gut in the experimental fish shows no physical changes in the four treatments 1(plate 9), followed by Treatment 2(plate12), Treatment3 (plate15), and Treatment 4(plate18) this is similar to the findings of the present work mentioned by (Hussein *et al*. 2000), (Soliman *et al*. 2000) and (Abdelhamid *et al*. 2002 b and Abdelhamid *et al*. 2002c).

VII. Conclusion

The result shows that ginger base diet with 69.7ppb aflatoxin shows reduce survival and 10% inclusion has the highest survival rate. The present result shows no damage in gills and gut with mild diffuse in hepatic vacuolar of two treatments in the liver. The significance of the research work to farmers is that any stored feed that is moulded can still be use with the inclusion of ginger up to 10% to reduce the effect of aflatoxin on fish tissues.

VIII. Recommendation

It is recommended that this study could be researched further to improve moulded aquacultural feeds. Further research should be conducted on blood parameters and tissues with marginal inclusion level of 15% to ascertain the most appropriate ginger inclusion on mould feeds.

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