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Effect of Aflatoxin B1 on growth performance of *Clarias Gariepinus* Fry (Burchell, 1822) in West Cameroon

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Key words: Aflatoxin B1, Chicken dropping, *Clarias gariepinus*, Fry, Growth performance

Abstract

The high cost of fish feed these days leads the developing countries such as Cameroun to use inexpensive and cheap local by-products. However, their source and or their conservation could generate mycotoxins affecting the performances of fish production. This work was carried out from January to June 2014 at the teaching and research farm of the University of Dschang, consisted to evaluate the effect of local feed contaminated with aflatoxine B1 on the production performances of *Clarias gariepinus*. To do this, 270 fry (4±2g) distributed in three treatments T1 (10 ppb), T2 (17 ppb) and T3 (20 ppb) and in triplicat (30 fry/tank of 1m3) and feeded of 5% of lichtyobiomass-fed daily were used. The high source of aflatoxin B1 in each treatment was from contaminated chicken dropping. Aflatoxine B1 has negatively affected (P<0,05) the growth characteristics regardless of its rate in the food. These effects increase with the rate of aflatoxin B1 contained in food. Moreover, the contamination rate at 10 ppb could be acceptable despite its overweight K (0,40±0,20) because this treatment show the best growth performances (weight Gain (35,40±11,62g), Specific Growth Rate (1,46±0,24), Consumption Index (2,01±0,7)). No significant effect of aflatoxin B1 bio-accumulate in the fish tissue was observed, it is same for the hematologic parameter considered and survival. The presence of aflatoxin B1 in the local feed affects growth of *Clarias gariepinus* fry and the extension of this study on fish reproduction could significantly improve fish production.

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Introduction

Reduced landings of fishery products and increasing demand in relation to the population explosion are a few reasons why the fishing cannot be a sustainable source of fish supply (FAO, 2016). That's why great interest is granted to fish farming, which is the fastest growing animal food producing sector in the world (Thomas *et al*., 2017). Nevertheless, the food happens to be a limiting factor for its emergence. Furthermore, the study on local-product and animal wastes such as chicken droppings in fish feed proved convincing. Due to their richness in protein, the chicken droppings can serve as an ingredient in the local fish food or as direct feed (Adewumi, 2011). They can replace 60% of soybean meal in the diet of *Clarias gariepinus* without any negative effects on growth (Obasa *et al*., 2009). However, poor conservation of these droppings as any other ingredient or so the fact that they reflect the chickens feed can be a source of contamination with mycotoxins including aflatoxin B1. Aflatoxins are toxins that belong to the group of mycotoxins, which are naturally produced by certain fungi, mainly by *Aspergillus flavus* and *Aspergillus parasiticus* (Milita *et al*., 2010; Andleeb *et al*., 2015). The food of chicken generally contains oilseed cakes that are potential sources of aflatoxin production when not properly stored. The aflatoxin surplus that could not be absorbed by the liver of chicken could be eliminated through faces which will later be used for fish and can cause high mortality in aquaculture (Dirican, 2015).

The resultant effect of aflatoxin on fish feed will lead not only to high cost of production but also, decrease in total farm production (Sotolu *et al*., 2015). Several studies revealed that Aflatoxin B1 residues can be retained in aquatic animal tissues, giving rise to potential public health risks after ingestion (Santacroce *et al*., 2011). Moreover, the presence of aflatoxins decrease the nutritional value of administrated feed in fish farm, both affecting the fish welfare status and the product quality (Hassan *et al*., 2010).This toxin may have more or less significant impacts on fish production performance (Ogunjobi *et al*, 2012 ; Agbebi *et al*, 2012; 2013).

This work aims to improve fish production through controlling the quality of local food used in African catfish (*Clarias gariepinus*). More specifically, it came to assess the effect of local food containing chicken droppings contaminated with aflatoxin B1 on growth performance, the accumulation of aflatoxin B1 in the flesh, some hematological parameters and survival of fry.

Material and methods

Site of the study, diets and experimental procedure

The study was conducted in the Application and Research Farm (FAR), Animal Physiology and Animal Health Laboratories of the University of Dschang located at 5°36'- 5°44' NL, 9°94'- 10°06' EL and at an altitude of 1400 m in the Western region of Cameroon.

The climate is Sudano-Guinean altitude type and includes a rainy season (mid-March to mid-November) and a dry season (mid-November to mid-March). Annual average temperature and rainfall are 22°C and 1800 mm respectively.

Given its hardiness and its availability in all fish farms in the region, *Clarias gariepinus* was selected to conduct this study. 270 fingerlings (4 ± 2 g) were collected for artisanal Hatchery GIC-AIO *"Groupement d'Initiative Commune Aquaculture Intégrée de l'Ouest"* of Batie in the highlands of West Cameroon and transported in hatcheries. These fry were divided into three homogeneous groups after a adaptation period of 2 weeks. During this period, they were fed ad libitum with a standard diet (3A) consisting of wheat flour, soybean meal and fishmeal (Lacroix, 2004).

The experimental diets contained the chicken droppings as ingredient (Table 2) contaminated with 5; 7.2 and 8.2 ppb aflatoxin B1 based on the diets fed to chickens. They were collected at the teatching and research farm of the University of Dschang, sun dried, ground and incorporated into rations. The aflatoxin B1 contained in the food of fish after analysis was 10, 17 and 20 ppb for treatments T1, T2 and T3, respectively (Table 1).

Table 1. Rate of aflatoxin B1 in chicken droppings and food (ppb).

Treatments	aflatoxin quantities in droppings (ppb)	aflatoxin quantities in food (ppb)
T1	5	10
T2	7,2	17
T3	8,2	20

Table 2. Composition of experimentals diets.

Ingrédients (Kg)	(%)
chicken droppings	20
Maize	7
Weath bran	8
Cotton cake	7
Soy meal	25
Groundnut meal	5
Fish meal	20
Shell meal	2
Bone meal	1
Palm oil	3
Premix 2%	2
Total	100
Chemical composition (% / MS)	
Crude protein	45,63
Gross energy (Kcal/kg)	2468
Crude fiber	4,8
Calcium	2,5
Phosphorus	1,2

Each 2.5 kg of 2% Premix (vitamins and minerals) contrains: vitamine A = 12.5 MIU (million international unit); vit D = 3.25 MIU; vit E = 40g; vit K3 = 2g; vit B2 = 5.5 g; vit B6 = 5g; vit B12 = 0.25 g; Niacin = 55g; Calcium pantothenate = 11.5 g; choline chloride = 500g; Folic acid = 1g; Bioline = 0.08 g; Manganese = 120g; iron = 100g; Zinc = 80g; Copper = 85g; Iodine = 1.5g; Cobalt = 0.3 g; Selenium = 0.12 g; Antioxidant = 120g.

All the treatments was randomly assigned to 3 experimental units repeated 3 times with 30 fry per repetition thus 90 fry per treatment. The trial was conducted in 9 concreted tanks (1m^3) filled with spring water with a revenge of 25 cm and covered with mesh. 25% of volume of water was renewed every 3 days. The food was distributed to fish at 8am and 6pm in powder form and representing 5% of ichtyobiomasse during study period (5 months). 20% of fish from each treatment were randomly taken each month, measured and weighed individually respectively by a ichtyometer and SF-400 balance (1g precision). This allowed us to evaluate the fry growth performance and adjust the amount of food to distribute. The uneaten food were collected through a bowl placed on the tank bottom and a frame floating on the surface of the water to avoid the dispersion of the food on the surface of the tank and guide the uneaten food in the bowl.

Growth parameters and biochemical analyzes

Growth parameters

The following production parameters were determined:

- Food Consumption (g)

$FC = Fd - Re$ (1)

FC: food consumption, Fd: food distributed, Re: refusal

- Weight gain (g)

$wg = wf - wi$ (2)

Wf: final average weight, Wi: initial average weight;

- Average daily gain (g.d^{-1})

$ADg = {}^{wg}/_{T}$ (3)

Wg: Weight gain (g), T: duration of the assay (day);

- Specific growth rate (%. d^{-1})

$SGR = \frac{[\ln(wf) - \ln(wi)]x100}{T}$ (4)

wf: final average weight, wi: initial average weight, T: duration of the assay (day);

- Consumption Index

$CI = {}^{FC}/_{wg}$ (5)

FC: food Consumption, wg: weight gain

- Survival rate (%)

$Sr = \frac{Nf}{Ni} x\ 100$ (6)

Nf: final number of fish, Ni: Initial number of fish;

- Condition K factor (%)

$K = \left({}^{W}/_{L^3}\right) x\ 100$ (7)

W : fish weigth, L : total length

Biochemical analysis

At the end of the study, the fish blood was taken at the heart level following a method described by Baron (1968) and MPO "*Ministère des Pêches et des Océans*" of Canada (2004). Blood samples were analyzed at the Animal Physiology Laboratory of the University of Dschang.

Haematological parameters (red and white blood cells, lymphocytes, monocytes, granulocytes, platelets) were quantified using a veterinary hematology Genius brand (Model KT-6180, S / N 701 106 101 557).

The aflatoxin B1 (AFB1) analysis took place at Animal Health Laboratory of the University of Dschang. The principle was based on the reaction between antigen (molecule to be measured) and antibody to form an antigen - antibody complex. The AFB1 assay kit provided by the company "Helica Biosystems Inc." consisted of a micro-well, 6 aflatoxin B1 standard solutions of different concentrations (0.0, 0.2, 0.5, 1.0 , 2.0 and 4.0ng/ml) and their enzyme conjugates, antibodies, and a stop solution. The assay method was that recommended by the catalogue of the kit. The optical density (OD) of the solution was measured at 450 nm using a brand ELISA reader Labsystems multiskan RC 6.0. From the DO standard, a calibration curve was plotted and allowed to determine the concentrations of AFB1 (ppb) of faeces, food and flesh of fish.

Statistical Analysis

The data on growth and biochemical parameters were subjected to analysis of variance (ANOVA) at one factor.

When there were significant differences between the averages, they were separated by Duncan test at 5% significance level. The SPSS 20.0 statistical software was used for these analyzes.

Results

Effect of aflatoxin B1 in feed on growth parameters of Clarias gariepinus fry

Table 3 show that the weak growth performance have been recorded in fish from treatments t2 and t3 in which aflatoxin b1 rate (17 ppb and 20 ppb) were higher than the t1 treatment (10 ppb). The anova revealed no significant difference ($p>0.05$) between t1 and t3 treatments for feed consumption (table 3). the largest live weight, weight gain, specific growth rate, total and standards lengths were recorded in the t1 (10 ppb) treatment. These characteristics tend to decrease with the increasing rate of afb1 in food ($p<0.05$). The better consumption index ($p<0.05$) was obtained with the treatment t1. Condition factor k was weak ($p<0.05$) in all treatments and fish survival was not significantly affected ($p>0.05$) by afb 1.

Table 3. Growth characteristics and survival of *Clarias gariepinus* in function of treatments

Growth characteristics	Rations			
	T1 (n=30)	T2 (n=30)	T3 (n=30)	P
FC (g)	39±0,00^{a}	28±0,00^{b}	39±0,00^{a}	0,000
Live weight (g)	39,85±12,00^{a}	25,00±7,73^{b}	23,80±7,93^{b}	0,000
WG (g)	35,40±11,62^{a}	20,65±8,11^{b}	19,45±8,71^{b}	0,000
ADG (g/j)	0,25±0,08^{a}	0,15±0,058^{b}	0,14±0,06^{b}	0,000
TL (cm)	17,38±4,53^{a}	15,48±1,38^{b}	15,30±1,66^{b}	0,050
SL (cm)	15,55±2,06^{a}	13,67±1,22^{b}	13,55±1,65^{b}	0,000
SGR (%)	1,46±0,24^{a}	1,16±0,31^{b}	1,16±0,40^{b}	0,007
CI	2,01±0,70^{b}	3,79±1,40^{a}	3,75±1,83^{a}	0,000
K	0,40±0,20^{b}	0,50±0,20^{a}	0,50±0,20^{ab}	0,069
Survival rate (%)	100	100	100	-

a, b : the means with the same letter on the same line are not significantly different (P>0,05), (n):number of animals P= Probability, SGR = Specific Growth Rate, TL = Total Length, LS = Standard length, FC = Feed Consumption, GMQ = Average Daily Gain,

Effect of aflatoxin b1 rate on feed consumption of clarias gariepinus fry

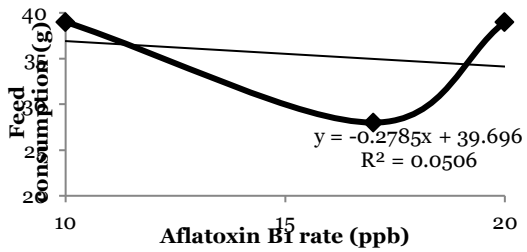


Fig. 1. Regression of feed consumption on aflatoxine B1 rate.

A significant decrease ($P<0.05$) of feed consumption was observed at treatment T2 of fish containing 17 ppb of aflatoxin B1 (AFB1) compared to the two other treatments T1 and T3 containing respectively 10 and 20 ppb of AFB1 (Fig. 1). However, according to the coefficient of determination ($R^2 = 0.050$) of the regression curve, food intake was weakly related to levels of AFB1 in food. The live weight of fish decreased significantly ($P<0.05$) with increasing rate of aflatoxin B1 (Table 3).

The curve of monthly evolution of body weight in function of the levels of AFB1 in treatment shows a stability weight the first month (Fig. 2) and an exponential growth of fish of treatment T1, then comes that of T2 and T3 treatments this until the end of the test.

Effect aflatoxin b1 rate on live weight of clarias gariepinus fry

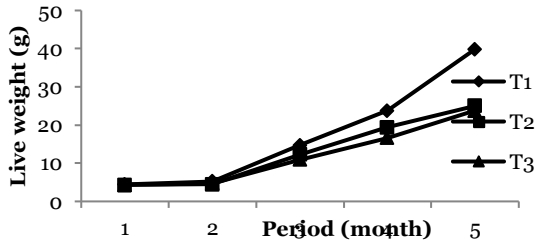


Fig. 2. Monthly evolution of live weight in function of aflatoxin B1 rate.

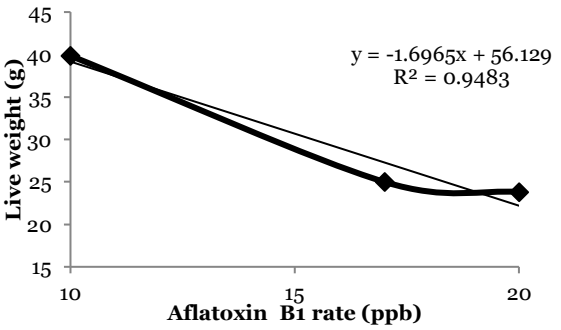
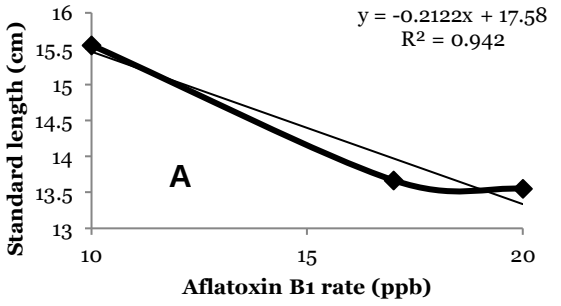


Fig. 3. Regression of live weight on aflatoxin B1 rate.

The shape of the regression curve (R^2 = 0.948) of body weight in function of AFB1 rate in feed (Fig. 3) shows a strong correlation between weight growth of the fish and the rate of this toxin in the food.

Effect of aflatoxin b1 rate on total and standard length of clarias gariepinus fry



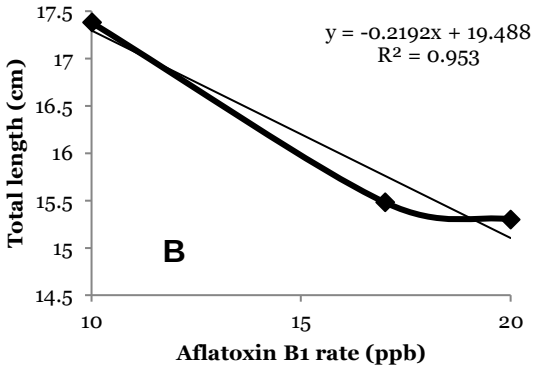


Fig. 4. Regression of total (A) and standard (B) length on aflatoxin B1 rate.

These Fig. shows curves that follow closely the regression lines with very high coefficients of determination (R^2 = 0.953 and R^2 = 0.942 respectively for the total length and standard length), reflecting a strong correlation between these characteristics and the rate of AFB1 in food.

Effect of aflatoxin b1 rate on specific growth rate of clarias gariepinus fry

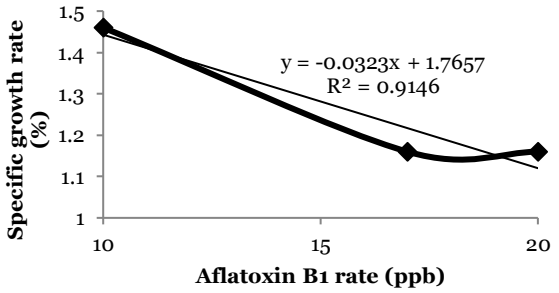


Fig. 5. Regression of specific growth rate on aflatoxin B1 rate.

Effect of aflatoxin b1 on consumption index of clarias gariepinus fry

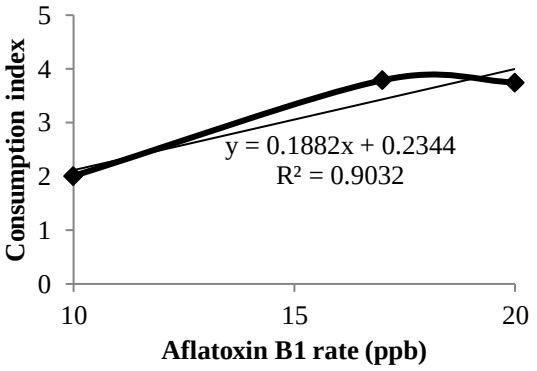


Fig. 6. Regression of consumption index on aflatoxin B1 rate.

The regression curve of the specific growth rate (Fig. 5) shows that this characteristic is highly correlated with the rate of AFB1 in the feed as indicated by the coefficient of determination (R^2 = 0.914).

The coefficient of determination (R^2 = 0.903) reveals that the consumption index was strongly related to the rate of AFB1. The condition factor has evolved as saw tooth over the entire period of the study and was less than 1 in all fish groups (Fig. 7).

Effect of aflatoxin b1 on condition factor k of clarias gariepinus fry

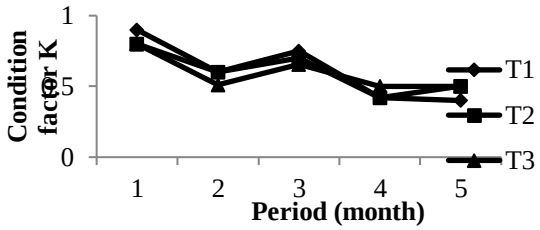


Fig. 7. Monthly evolution of condition factor K in function of aflatoxin B1rate

Effect of aflatoxin b1 in feed on haematological parameters of clarias gariepinus fry

Table 4. Hematological parameters of *Clarias gariepinus* fry in function of treatments

hematological	Rations				
Parameters	T1	T2	T3	P	Sig
WBC (×10*9/l)	37,45±8,75	41,07±20,61	38,10±15,20	0,647	NS
Lymphocytes (×10*9/l)	34,20±8,60	37,00±21,22	29,10±6,90	0,801	NS
Monocytes (×10*9/l)	1,65±0, 35	1,60±0,60	3,53±2,48	0,041	NS
Granulocytes (×10*9/l)	1,60±0,2	2,47±2,54	1,80±0,10	0,144	NS
RBC (×10*12/l)	2,00±0,40	1,68±0,51	1,30±1,00	0,712	NS
Hématocrit (%)	23,37±5,08	21,20±4,62	23,68±2,52	0,629	NS
MCV (fl)	117,30±5,73	129,00±10,67	135,10±8,75	0,479	NS
PLT (×10*9/l)	511,33±18,82	524,00±168,00	570,66±194,74	0,868	NS
PDW(%)	8,07±2,60	8,55±3,45	11,53±4,14	0,909	NS
PCT(%)	0,41±0,06	0,43±0,19	0,71±0,85	0,908	NS

NGB = Nombre de lobule Blanc, NGR = Nombre de Globule Rouge, MCV = Mean Corpuscular Volume, fl = fento liter, PLT = Platelets, PDW=Platelets Distribution Width, PCT=Plaquettocrite, Sig = signification, NS = Not Significant, P = Probability.

The food contaminated with AFB1 had no significant effect (P>0.05) on hematological parameters of fish (Table 4). However, the number of white blood cell was higher in T2 and T3 treatments compared to T1 while the number of red blood cells decrease as the AFB1 rate increases in the food.

Bio-accumulation of aflatoxin b1 in flesh of clarias gariepinus fry

Table5. Accumulation of aflatoxin B1 rate dans in flesh of *clarias gariepinus* fry in function of treatments.

Parameters	Rations				
	T1	T2	T3	P	Sig
Aflatoxin B1 accumulated (ppb)	0,05±0,12	0,08±0,10	0,08±0,12	0,842	NS

Sig = Signification, NS = Not Significant, P = Probability.

The rate of AFB1 accumulated in the flesh of fish was very small but not significant (P> 0.05) between treatments (Table 5).

Discussion

The weak growth performance have been recorded in fish from treatments T2 and T3 in which aflatoxin B1 rate (17 ppb and 20 ppb) were higher than the T1 treatment (10 ppb). The best daily gain and specific growth rate (P <0.05) were obtained in fish of treatment T1 (10 ppb). The small value of these characteristics in treatment who receive high level of AFB1 can be explain by a lower digestibility of feed of these treatments. Average daily gains in this study are lower than 3 g/day obtained by Lacroix (2004). It is the same for specific growth rate that are lower than those obtained by Ruby *et al.* (2013) in *Labeo rhohita*. On the contrary, these values are almost identical to those obtained by Ogunjobi *et al.* (2012) in *Clarias gariepinus* fed food contaminated with aflatoxin B1.

The results indicate that *Clarias gariepinus* fry least appreciate the food containing AFB1. Nevertheless, they better appreciate the food with low AFB1 although the presence of this toxin in food reduces the appetite. The lowest consumption index ($P < 0.05$) was obtained with the treatment T1 (10 ppb). This result shows that the treatment T1 (10 ppb) has been better valorise by *Clarias gariepinus* fry compared to treatments T2 (17 ppb) and T3 (20 ppb). This may be due to the fact that high level of aflatoxin B1 reduce the appetite of fish. These results corroborate those of Ogunjobi *et al.* (2012) who reported that the consumption index of the control was less than those of treatment containing higher AFB1 in food.

Condition factor K has evolved significantly ($P<0.05$) as saw tooth over the entire period of the study and was less than 1 in all fish groups. However, according to Fulton (1902) this result indicates that the fish were in poor physical condition during the test. In this study, the food contaminated with AFB1 had no significant effect ($P>0.05$) on hematological parameters of fish. However, the number of white blood cell was higher in T2 (10 ppb) and T3 (20 ppb) treatments compared to T1 (10 ppb). This may be because the body of the fish produced antibodies to defend against AFB1. This result is similar to that reported by Dahunsi and Oranusi (2013) who found in *Clarias gariepinus* exposed to an increasing level of rubber effluent a high number of white blood cells. These authors explain this result by an attempt to fight against antigens (pollutant) by fish. However, we observe a decrease number of red blood cells as the AFB1 rate increases in the food. This could be explained by lysis of the red blood cells by AFB1. Martinez and Souza (2002), Atamanalp Yanik (2003) and Abdel-Hadi *et al.* (2011) reported similar results in fish exposed to the effluent containing a high level of toxin.

The rate of AFB1 accumulated in the flesh of fish was very small but not significant ($P>0.05$) between treatments (Table 5). Mahmoud *et al.* (2014) reported similar results in the *Lates niloticus*. The traces of AFB1 in the flesh can be explained primarily by the small amounts of food ingested. This implies that the quantity of AFB1 ingested through the food was not important. Secondly, the body detoxification mechanism could also facilitating excretion of this toxin and prevented greater accumulation in the muscles. Indeed, the amounts of AFB1 ingested are absorbed by the gastrointestinal tract where they will be metabolized or detoxified in the mucosal cells (Frink-Gremmels, 1998). They may also be eliminated from the body through the urinary and biliary excretion (Richard, 1998).

Generally, fish survival was not significantly affected ($P>0.05$) by AFB1. Survival rates were high (100%) in all treatments. This result can be explained by a low amount of AFB1 consumed by the fish. Our results were different from that of Sepahdari *et al.* (2010) who reported mortality rate of 55% after 6 weeks and 40% after 8 weeks in fish fed with contaminated food by 100mg of aflatoxin B1/kg of feed and Ogunjobi *et al.* (2012) who had a mortality rate of 60% in *Clarias gariepinus* fed a diet containing moldy corn as an ingredient.

Conclusion

The growth characteristics were significantly affected by aflatoxin B1. Feed consumption was significantly lower with the diet contaminated with 17 ppb of aflatoxin B1. Body weight, weight gain, total length, standard length and specific growth rate of the fish exposed to treatment T1 contaminated with 10 ppb of AFB1 were significantly higher while the consumption index and condition factor K were significantly lower with the same treatment. Although there has been no significant difference in hematological parameters, the presence of aflatoxin B1 in feed resulted in a not significant increase of white blood cell and and reduction of red blood cells number. Fish survival was not affected in all treatments. Whatever the levels of aflatoxin B1 in feed, the amounts accumulated in the flesh of fish were very small. Feed with 10 ppb of aflatoxin B1 is better tolerate by *Clarias gariepinus* fry.

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