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ABSTRACT

The study investigated oxidative stress induced by Aflatoxin B1 (AFB1) in selected palaemonid shrimps. The shrimps were fed with Aflatoxin B1 of 0, 2, 5, 10, 20, and 40 µg/kg in mixtures of shrimp feed. The impact s of the concentrations on the growth rate of the shrimps and the induced Oxidative stress were assessed. Growth rate was estimated using standard growth rate, mean weight gain, feed conversion rate and survival rate; while oxidative stress was estimated using levels of Superoxide dismutase (SOD). Generally, AFB 1 of 40 µg/kg bw highly impacted the growth of all species, followed by 20 µg/kg bw. i.e. the shrimps in the treatment elicited significantly reduced standard growth rate and mean weight gain. Elevated concentrations of SOD detected in all shrimp species at AFB1 concentration of 20 µg/kg bw, particularly 40 µg/kg bw further supported the toxicity observations in the treatments. According to the profile of antioxidant activities and growth analysis, the trend of resilience to AFB 1 was: *Macrobrachium vollenhovenii* >*Macrobrachium macrobrachion* >*Macrobrachium dux* >*Macrobrachium fellicinium* > *Palaemon maculatus* > *Nematopalaemon hastatus*. This implies that *M. vollenhovenii* followed by *M. macrobrachium* have higher success potentials in small and middle scale shrimp farms vulnerable to aflatoxin exposure. Palaemonid shrimp farm feeds should be regularly screened to ensure the concentration of AFB1 does not exceed 10 µg/kg bw.

Keywords: Palaemonid shrimps, oxidative stress, Aflatoxin B1, success potential, shrimp farm

1. INTRODUCTION

Aflatoxins belong to a group of toxins called Mycotoxins, which are diverse fungal metabolites. Fungi are ubiquitous and those which produce Aflatoxins include *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin B1 (AFB1) and several aflatoxin analogues are secreted by these fungi in stored dairy feeds (Ashiq, 2015). Aflatoxins B1, B2, G1 and G2 are ubiquitous mycotoxins of farm feeds (Lourduraj *et al*., 2017). However AFB1 has generated the most research interest, owing to the fact that it has the highst toxicity potential in the group. AFB1 is a

highly toxic and carcinogenic mycotoxin to many animal species (Tiwari and Rana, 2015). It has been reported in many literatures that AFB 1 elicits significant hepatotoxicity, nephrotoxicity and immunotoxicity in exposed farm animals, which may culminate in poor farm productivity (Santos *et al.,* 2010; Adeyemo *et al.,* 2016).

One of the problems confronting small and middle scale shrimp farmers are related to feed invasion of Aflatoxin (Cary *et al.,* 2011). Impacts of mycotoxins on fish and shellfish production have been a global topical discourse, as fish and shellfish constitute an important part of global diet (Factfish, 2018).

It has been reported that approximately 200 different fungal species produce about 300 mycotoxin (Wei *et al*., 2017). The toxigenic fungi that produce mycotoxins are abundant in the soil. Consequently, agricultural products, being inevitably associated with soil are highly susceptible (Cary *et al.,* 2011). Factors which foster susceptibility of agricultural products are a wide range of environmental stressors, particularly during growth. These include, high temperature, low humidity, acidic conditions and other environmental factors in which fungi thrive (Pitt *et al.,* 2000). Other factors include poor storage conditions of farm products (Bankole and Adebanjo, 2003) which are prevalent in developing countries with poor storage facilities such as Nigeria.

Worse events in which AFB 1 inflicted teratogenic and mutagenic effects and even death of both farm animals and humans have been widely reported (Quintana *et al.,* 2012). Toxicity of AFB 1 is a function of many factors which include species of the exposed organisms, age, sex, nutritional and or health status as well as concentration and duration of exposure (Tola and Kebede, 2016). Shrimp entrepreneurship is an emerging industry in the Nigerian economy. Even at its infancy, it is not exempted in the challenges of Aflatoxin B1 toxicity in shrimp feeds (Ahmed *et al.,* 2016). The current study is based on the previous physical observations of Wei *et al* (2017) that based on growth performance, pre-adult shrimps appeared to tolerate AFLB1 concentration of up to52.3 µg/kg administered orally through feed mixtures. However, they observed changes in the tissues of some shrimps given diets of 26.5µg/kg AFLB1. This clearly indicates toxicity is possible even at lower concentrations than have been observed.

Therefore, this study aims at comparative determination of concentrations of AFLB1 below and above 26.5 µg/kg AFLB1 observed in the previous study to further ascertain the impacts on the growth performances (Akinwole and Faturoti, 2006) and oxidative stress across the range.

2. MATERIALS AND METHODS

2.1. Collection and identification of shrimps

Viable palaemonid shrimps obtained from Osse River, in Edo State, Nigeria were identified to species level using identification manuals such as FAO (1981) and Powel (1982). Shrimps were identified as *Macrobrachium macrobrachion* (6.14±0.3g), *Macrobrachium vollenhovenii* (10.2±3.1g), *Macrobrachium felicinium* (7.2±2.2g), *Macrobrachium dux* (4.8±0.12g), *Nematopalaemon hastatus* (5.5±0.6g) and *Palaemon maculatus* (4.2±0.4g).

All experimental procedures were conducted in compliance with the guidelines provided by National Animal Ethics Advisory Committee (2010). Procedures also conformed strictly to the stringent guidelines provided by the Institute for Laboratory Animal Research (2010).

2.2. Acclimatization

Shrimps were inspected for general fitness prior to acclimatization. Unfit fish individuals were discarded while viable individuals were acclimatized for 14 days under natural day and night photoperiods (12/12-hrs) before the commencement of the experiment in 6 aerated 96 L glass aquaria (55x72x72cm). The shrimps were fed ad-libitum with shrimp feed; supplemented with cassava. The physico-chemical conditions of the water were 26- 28 ^{o}C, 5- 8.5 mg/L (DO); both assessed with the aid of a Model JPSJ-605 DO-Analyzer, and 6.8 – 8 (pH) measured using the Electric Probe Hydro-lab water quality meter (HANNA HI 9813 GRO).

2.3. Diet preparation and feeding regime

AFB1 mixed diets were prepared by adding 1 mg crystalline research grade AFB_1 to 1 mL chloroform to prepare a mixture of 1 mg: 1,000 µL aliquot of AFB1. Then, required quantities of the solution to produce the various concentrations of diets were pipetted in to 100 mL volumetric flasks and volume made up to the 100 mL using methanol. A prepared aliquot of 1 gram AFB_1 was dissolved in 1,000 µL of acetonitrile-water (v:v) (resulting in 1 µL:1 mg solution of fumonisin B1). The feeds were then added to the liquid mixture of AFB1 to produce the different diet concentrations: 0 µg AFB1/kg bw (control), 2, 5, 10, 20, and 40 µg AFB1/g bw. The feed mixtures were then blended, pelletized and fed to *M. macrobrachion*, *M. vollenhovenii*, *M. felicinium*, *M. dux*, *N. hastatus* and *P. maculatus;* 10 representatives in each of 6 aquaria for 36 days.

2.4. Oxidative stress

2.4.1. Preparation of post-mitochondrial supernatant (PMS)

Shrimps were retrieved from the aquarium and sedated with 40 % methanol. Afterwards, they were stripped of their exoskeleton, telson, mouth parts and other hard cuticles. Samples were then rinsed with distilled water, weighed and thawed in at –10 ^{o}C. Whole tissue was homogenized in chilled TRIS buffer (100 mM, pH 7.8; 1:10 w/v); with the aid of an Ultra-Turrax tissue homogenizer. Afterwards, the homogenates were centrifuged at 10,500×*g* for 20 mins at 4 ^{o}C to obtain the post-mitochondrial supernatant (Graham, 2002).

2.4.2. Analysis of superoxide dismutase

Superoxide dismutase (SOD) concentration was measured using the xanthine oxidase–cytochrome method as demonstrated by McCord and Frodovich (1969). The assessment followed the reaction of Xanthine with 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride to form superoxide radicals which further reacted to forming a reddish formazan; which was marked as an indicator of SOD induction in the tissues. The mode of action of SOD in reducing availability of superoxide radicals inhibited formation of formazan. Inhibition percent of formazan formation was then assessed with the aid of spectrophotometer at 505 nm and this signified the rate of induction of SOD.

2.5. Analysis of growth and survival indices

The effects of mixed feed on growth performance were determined by evaluating the Specific Growth Rate (SGR), Mean Weight Gain (MWG), Feed Conversion Ratio (FCR), and Survival Rate (SR).
The specific growth rate was ascertained thus:

$$SGR = \frac{lnW2 - lnW1}{t2 - t1} \; X \; 100 \;\; (Solomon, 2006)$$

Where W_2 is weight of shrimp after period of feeding on diet; W_1 is weight before the feeding regime; t_2-t_1 is the duration (in days).

Mean weight gain was calculated thus:

$$MWG = \frac{Final\ mean\ weight - Initial\ mean\ weight}{Initial\ mean\ weight} \; X \; 100$$

Feed conversion rate was calculated thus:

$$FCR = \frac{Weight\ gain}{Feed\ intake} \; X \; 100$$

Survival rate was calculated thus:

$$SR = \frac{Total\ shrimp\ retrieved}{Total\ shrimp\ recruited} \; (\text{Akinwole } and\ Faturoti, 2006)$$

2.6. Statistical analysis

Results obtained were presented as the mean ± S.E. The differences in Specific Growth Rate (SGR), Mean Weight Gain (MWG), Feed Conversion Rate (FCR), Survival Rate (SR) and Superoxide Dismutase (SOD) induction after 36 days of feeding among the different species of shrimps were analyzed by two-way Analysis of Variance (ANOVA) and ascertained by Duncan Multiple Range (DMR) test ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Impacts of AFB1 on growth indices

M. vollenhovenii followed by *M. macrobrachium* exhibited significant SGRs in control and 2µg/kg bw treatments. This implies that these species have higher growth rates than other palaemonid species. This observation conforms to earlier observations (Isibor, 2016). Generally, *P. maculatus* and *N. hastatus* had the lowest MWGs (Table 1). The MWGs recorded in both species were extremely low in the treatments with 20 µg/kg bw and 40 µg/kg bw ($p < 0.05$). Results indicates that *M. vollenhovenii* and *M. macrobrachium* were least impacted, while *P. maculatus* and *N. hastatus* were most impacted by AFB1 in terms of growth indices.
A downward trend was observed in the SGRs and MWGs as concentrations of AFB1 increases from control treatment towards 40 µg/kg. Shrimps fed 40 µg/kg were most impacted, followed by those fed 20 µg/kg. Significant impact on SGR of *M. vollenhovenii, M. macrobrachium, N. hastatus,* and *M. dux* began from AFB1 of 10 µg/kg and above ($p < 0.05$). Significant impacts ($p < 0.05$) on MWG was also observed from 10 µg/kg and above in all the shrimp species. This

indicates that AFB1 of 10 µg/kg significantly impacted the development of the experimented shrimps. The highest impacts were however observed in shrimps fed 40 µg/kg AFB1.

Table 1: Impacts of AFB1 on shrimp SGR and MWG

Shrimps	Control		2µg/kg		5 µg/kg		10 µg/kg		20 µg/kg		40 µg/kg	
	SGR	MWG	SGR	MWG	SGR	MWG	SGR	MWG	SGR	MWG	SGR	MWG
MV	**2.32**^{A}	**112.2**^{A}	**2.01**^{A}	**104.4**^{A}	**1.82**^{A}	**102.5**^{A}	1.61^{A}	77.3	0.62	12.4^{A}	0.07	2.01
MM	**1.44**^{B}	**110.4**^{A}	**1.04**^{B}	**102.4**^{A}	**1.40**	**97.4**	0.34^{A}	63.4	0.32	18.6^{A}	0.47	-0.23^{A}
MD	0.54	**102.2**^{A}	0.41	**92.4**^{B}	1.02	**87.6**	**0.66**	69.3	**0.01**	**0**	**-0.2**^{A}	**0.12**
MF	0.23	**90.4**^{B}	0.54	**87.2**^{B}	0.40	**84.3**	0.34	18.2	0.22	5.8	**-0.45**^{A}	**0.55**
PM	0.15	**78.4**^{B}	0.21	**57.3**	0.22	**54.2**	0.16	12.7	**0.006**^{A}	**0**	0.23	**-0.23**^{A}
NH	0.41	**81.4**^{B}	0.34	**68.2**	0.31	**61.8**	0.21	**4.4**^{A}	**0.07**	**-1.2**	**0.02**	**-2.21**^{A}

KEYS: MV= *Macrobrachium vollenhovenii,* MM= *Macrobrachium macrobrachion,* MD= *Macrobrachium dux,* MF= *Macrobrachium fellicinium*, PM= *Palaemon maculatus*, NH= *Nematopalaemon hastatus,* SGR= Standard Growth Rate, MWG= Mean Weight Gain. NOTE: Figures with different alphabets mean significant difference across species while emboldened figures show significant difference across concentrations ($p<0.05$).

3.2. Impacts of AFB1 on survival chances

Significant SGRs earlier observed in *M. vollenhovenii* and *M. macrobrachium* in the control and AFB1 2µg/kg treatments can be attributed to significant ($p< 0.05$) FCRs in the treatments (Table 2). The FCRs of *M. vollenhovenii* and *M. macrobrachium* were impacted at 5 and 10 AFB1 µg/kg respectively (Table 2).

Very low FCRs were observed in *M. felicinium, P. maculatus,* and *N. hastatus* in treatments with AFB1 20 and 40 µg/kg. SR of almost all the species significantly dropped at AFB1 10 µg/kg, except for *P. maculatus* and *N. hastatus* who's SR significantly reduced at lower concentration of 5 µg/kg.

Table 2: Impacts of AFB1 on shrimp FCR and SR

Shrimps	Control		2µg/kg		5 µg/kg		10 µg/kg		20 µg/kg		40 µg/kg	
	FCR	SR	FCR	SR	FCR	SR	FCR	SR	FCR	SR	FCR	SR
MV	**4.32**^{A}	**100**	**4.01**^{A}	**100**	2.82^{A}	**100**^{A}	0.11	84	0.42^{A}	84^{A}	0.02	41^{D}
MM	**3.44**^{B}	**100**	**2.04**^{B}	**100**	**3.20**^{A}	**100**^{A}	0.22	77	0.02	76^{A}	0.07	38^{D}
MD	0.54	**100**	2.41	**100**	1.32	**87.6**	0.66	69	**-0.01**	66	-0.62^{A}	33^{D}
MF	0.23	**100**	0.54	**100**	0.20	**84.3**	**0**^{A}	68	**0**	66	**0**	28^{C}
PM	0.12	**100**	**3.21**^{A}	**100**	0.12	54.2	0.16	68	0.2	67	0	13^{A}
NH	0.41	**100**	0.14	**100**	0.81	61.8	0.21	67	**0**	67	**0.01**	16^{A}

KEYS: MV= *Macrobrachium vollenhovenii,* MM= *Macrobrachium macrobrachion,* MD= *Macrobrachium dux,* MF= *Macrobrachium fellicinium*, PM= *Palaemon maculatus*, NH= *Nematopalaemon hastatus,* FCR= Food Conversion Rate, Survival Rate= Survival Rate. NOTE: Figures with different alphabets mean significant difference across species while emboldened figures show significant difference across concentrations ($p<0.05$).

3.3. AFB1-mediated oxidative stress

Elevated concentrations of SOD detected in all shrimp species at AFB1 concentration of 20 µg/kg bw, particularly 40 µg/kg bw further supports the toxicity observations in the growth analysis. This implies that the antioxidant defense system of the shrimps was highly induced into production of SOD to ameliorate the effect of the oxidative stress caused by AFB 1 at 20 and 40 µg/kg bw. Quintana *et al.* (2012) earlier reported stress inducing potentials of AFB 1. The least stressed at the highest concentration was *M. vollenhovenii*. Although *M. macrobrachium* coped better than *M. vollenhovenii* at 10 µg/kg bw, the former overtook the latter in SOD levels at higher AFB1 concentrations.

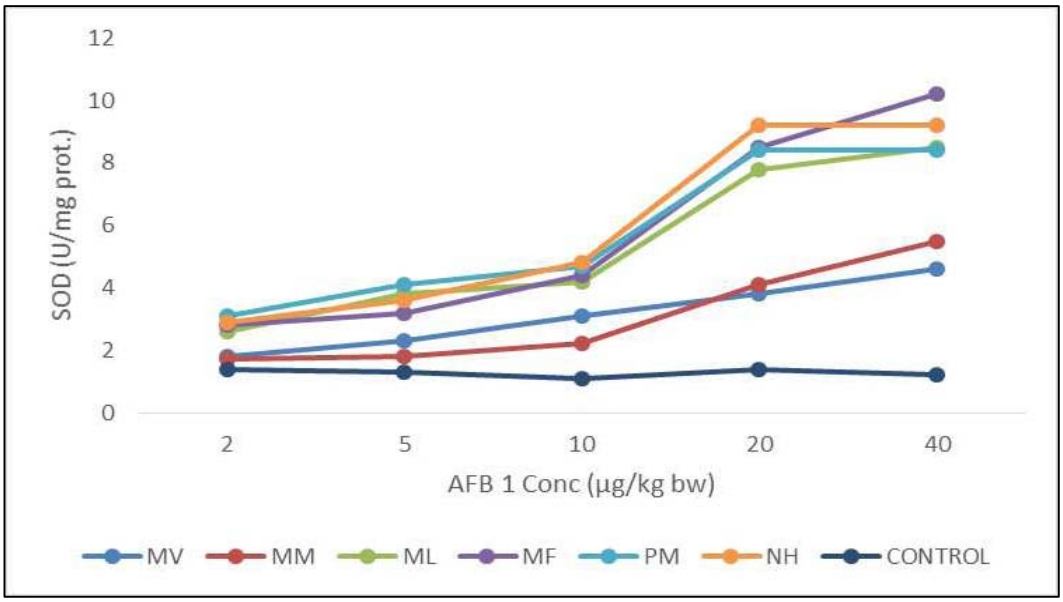

Figure 1: Superoxide dismutase (SOD) levels in different concentrations of AFB 1

4. CONCLUSION

AFB1 elicited toxicity potentials to the general wellbeing and survival of palaemonid shrimps. This was mainly observed at dosage of 10 µg/kg bw. A few sub-lethal impacts observed at 5 µg/kg bw indicate safe limit is below this concentration. However 5 µg/kg bw may be permissible. Results show that *M. vollenhovenii* followed by *M. macrobrachium* have significant success potentials in small and middle scale shrimp farms provided AFB1 concentration in feed is stringently regulated. We hereby recommend that palaemonid shrimp feeds should be regularly screened to ensure the concentration of Aflatoxin B1 in feed does not exceed 5 µg/kg bw.

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