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REGULAR ARTICLE

MYCOTOXINS CONTAMINATION IN EDIBLE LAND SNAIL AT GRAZING PADDOCK ENVIRONMENT

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ABSTRACT

Mycotoxins contamination of animal products is under reported. Juvenile edible land snails (*Archachatina marginata)* were exposed as sentinels in bottomless metal drums for 1 week at abandoned, new and reference sites respectively at grazing paddock environment, to assess the presence of foodborne microbiological mycotoxins contamination during the dry season. Mycological analysis of *A. marginata* samples revealed high ($p<0.05$) contamination at all paddocks ranged from 1.2-1.3 x 10^5 cfu^{-g}. Results revealed values that were found to be unacceptable by FAO/WHO standards. The presence of *Aspergillus niger, A. fumigatus* and *Penicillum expansum* were noted as potential toxicogenic mycoflora. Snails were tolerant to all levels of contamination with no clinical signs of infection or mortality. This finding could serve as basis for assessing pre-slaughter microbial contamination of livestock farm/field environment in order to establish data with comparative epidemiological value, which could highlight early warning signals of food safety risk and cross-contamination of mycotoxins in the food chain.

Keywords: Meat, mycoflora, microbiological contamination, food safety, edible mollusc

INTRODUCTION

Giant African land snails (GALS) are relished as delicacy by indigenes of Niger Delta region of Nigeria **(Ebenso and Ebenso, 2011).** Previous study provides evidence of edible snail as a bioindicator of transfer of foodborne (bacterial) pathogens within the food chain and for food safety assessment **(Ebenso *et al*., 2012).** Hence, edible snail provides early warning signal of toxins and pollutants in the environment **(Ebenso, 2012).**

Used as sentinels, snails are the representative primary consumers in the terrestrial ecosystem **(Naaem *et al*., 1994).** Snails are involved in many aspects of organic matter decomposition, potential regulation of microbial activities, nutrient cycles and crumby structures **(Cortert *et al*., 1999).**

Mycotoxins comprise a family of fungal toxins, many of which have been implicated as chemical progenitors of toxicity to man and animals **(Zaki *et al*., 2012).** Mycotoxins are regarded as extrolites (secondary metabolites of fungal origin) are odourless, tasteless and colourless **(Fapohunda, 2012).** Mycotoxins are toxic to humans and animals, which explains the major concern of food and feed industry in preventing them from entering the food chain **(Pierre, 2007).** Mycotoxins have been associated with a number of human diseases. **Beardall and Miller, (1994)** have given detailed account of human illnesses that have been associated with mycotoxin ingestion. Chronic intake is the most widespread form of human health exposure. **Bryden, (2007)** reported that the impact of regular low level of mycotoxins on human health is likely to be significant with the possible consequences including impaired growth and development, immune dysfunction and the disease consequences of alleviation in DNA metabolism.

Human food can be contaminated by mycotoxins at the various stages in the food chain from the genera *Aspergillus* (aflatoxins) and *Penicillum* (ochratoxins). The disease resulting from mycotoxin exposure is mycotoxicosis **(Bryden, 2007).** Human exposure may result from the carry over of mycotoxins and metabolites into animal products such as milk, meat and eggs **(CAST, 2003).** According to **Pestka, (1995)** trace levels of mycotoxins and their metabolites may carryover into edible tissue (meat) of food producing animals.

There is now overwhelming epidemiological evidence **(Wild and Hall, 1996)** that aflatoxin consumption contributes significantly to the high incidence of human liver cancer **(Henry *et al*., 1999)** and ochratoxin is nephrotoxic, a possible cause of urinary tract tumour **(Peraica *et al*., 1999).** Consumption of a mycotoxin contaminated diet may induce acute and long-term chronic effects in teratogenic, carcinogenic, and oestrogenic or immune –

suppressive effects. **Gong *et al*., (2002)** revealed a strong association between exposure to aflatoxin in children and both stunting and being underweight.

The objective of this study was aimed at using juvenile edible snail (*Archachatina marginata*) as sentinels at grazing paddocks to determine microscopic fungi involved in pre-slaughter contamination and to demonstrate potential adverse effects of mycotoxins within the livestock farm environment.

MATERIALS AND METHODS

Animal Management

The design of the experiment was completely randomized design. The experimental area was the Teaching and Research Farm, University of Uyo, within latitude $4^{o}31'$N and $4^{o}45'$N, longitude $7^{o}31'$E and $45^{o}51'$E with mean temperature of 30^{o}C and rainfall of 2000 – 3000mm per annum.

The 135 juvenile *A. marginata* snails 100±5.00g were randomly assigned to 3 treatment sites of grazing paddocks within the cattle unit namely, old (abandoned, no longer used for 1 year), new (currently in use) and reference (control) paddocks respectively, using 45 snails replicated 3 times.

The microcosm was bottomless metal drums (0.6m diameter and 0.6m high) with perforated lids for air, light and protects snails against predators. The snails as sentinels (from uncontaminated laboratory stock) were transferred to the bottomless microcosm (suitable for contact with soil and vegetation). These microcosms were positioned 20m apart per paddock. During the experimental period of 1 week in October (dry season), snails consumed food (soil and vegetation) *in situ* and *ad libitum*.

Sample Collection

Fresh snail samples for microbiological analyses were collected in labelled sterile isotherm polythene bags and then transported to the laboratory. The snails were extensively washed with water and rinsed with normal saline to remove all surface contaminants. The edible parts of the snails were dissected to remove internal extracts from the proximal gut for subsequent homogenization and serial dilutions.

Identification and Enumeration of Bacteria

One gram of snail extract was diluted serially in ten fold dilution blanks and properly mixed with sterile glass rod. The 0.1 ml of diluted sample was pipetted into sterile plate and molten sterile agar medium (45°C) was poured. The media used were plate count agar (PCA, Biotech), nutrient agar (NA Biotech), xylose lysine desoxycholate agar (XLD, Biotech) and DeMan Rogosa Sharpe agar (MRS, Biotech). The plates were rotated gently to disperse inoculum in medium and allowed to solidify. This was done in triplicates and plates were incubated at 37°C.

Mycoflora Identification

The snail extracts were transferred to a 0.9% saline solution (10 ml) and vortexed for 3 min. Then, 0.1 ml of this solution was transferred to Potato Dextrose Agar (PDA) plates. Later, the collected snail extracts were surface-sterilized by consecutive washing in sterile distilled water and 70% alcohol for 1 min (**Pereira *et al.*, 2009**). Each snail was immersed in 10 ml of phosphate saline buffer. Dilutions x1000 of each sample were seeded onto PDA plates. Plates were incubated at 28ºC.

Cultures growing on Potato Dextrose Agar (PDA) and Malt Extract Agar (Difco) were identified according to microscopic observations such as morphological characters of mycelium and conidia. Observations were made by staining the isolated fungus using lactophenol cotton blue and examination under low-power microscope. The species were identified according to **Von Arx, (1981); Hasenekoglu, (1991); Domsch *et al.*, (1993); Watanabe, (2002)**. The organisms were maintained on PDA slants at 4^0C.

Statistical Analysis

Enumeration of samples, with mycoflora (n=100), were performed in triplicates. Data were subjected to one way analysis of variance (ANOVA), using statistical analysis system **(SAS, 1992)**. Mean differences wer compared for statistical significance at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The *A. marginata* positioned as sentinels in metal drums in this study did not record any mortality or clinical infection; they were tolerant of high load of microbes. According to **Bryden, (2007)** the effect of immunity and resistance are often difficult to recognize in the field because signs of disease are associated with the infection rather than the toxin that predispose the individual to infection through decreased resistance. May be the experimental period of this study was not long enough for clinical signs in *A. marginata,* it was contrary to reports of **Bryden, (1982)** that available evidence suggests that tissue accumulation of mycotoxins or their metabolites was very low and that residues are excreted in a few days.

In Table 1, *E. coli* and *Salmonella* in *A. marginata* were not detected at the new and old paddocks. According to **Wilson *et al*., (2002)** *E. coli* and *Salmonella* are gram negative bacteria, and are infrequently recovered at feedlots, possibly because of their rapid inactivation by UV irradiation (from the sun). Reports of previous study by **Sproston *et al*., (2006)** with slugs have shown no relationship between *E. coli* and *Salmonella* carriage, as microbial analysis failed to detect pathogens. In fact, **Smith *et al*., (2001)** reported that the prevalence of *E. coli* and *Salmonella* in cattle was greater in paddocks that were muddy and wet. **Callaway *et al*., (2004)** indicated that detection of *E. coli* and *Salmonella* is also complicated by the fact that, fecal shedding can be very sporadic, with an animal testing positive one day, but not again for several days or even weeks**.**

Levels of mycotoxins in *A. marginata* in this study (Table 1), were higher ($p<0.05$) and unacceptable compared with FAO/WHO mycotoxins standards/levels in fresh meat **(JECFA, 2001)** consequently, the presence of moulds in meat and meat products could cause a decrease in their biological value due to the enzymatic degradation of meat components **(Okin *et al*., 2001).** According to **Zaki *et al*., (2012)** mycotoxins interfere with protein formation. **Kan and Meijer, (2007)** reported that aflatoxin binds both RNA and DNA and blocks transcription, while ochratoxin blocks RNA synthesize and thus blocks translation. Mycotoxins are non-antigenic, but an antibody response can be elicited to the toxin after conjugation to a protein or polypeptide carrier. In Table 1, the high total bacteria count is well above limit of 10^4 by **HPA, (2009); ICMSF, (1986).** Melabolic interactions of mycotoxins with bacterial pathogens has played important role in the outbreak of foodborne illnesses.

From analysed positive samples (Table 2 and 3), there were 9 fungi isolated from gut of *A. marginata*, of which *Aspergillus* and *Penicillum* are potentially toxigenic mycotoxins.

This compare with report of **Zaki *et al*., (2012)** that concurrent exposure to multiple mycotixins is more likely in the livestock industry. According to **Erber and Binder, (2004)** mycotoxin contaminations are due to combinations of two or more than two toxins. Co-occurrence of mycotoxins is of special concern, for instance, in the case of furmonisins (a potent cancer promoter) and aflatoxin (a potent human carcinogen) where a complimentary toxicity mechanism of actions occurs **(Riley, 1998).** In African and Asia, the co-occurrence of these mycotoxins is common and a significant percentage of the population is infected with Hepatitis B or C which leads to the conclusion that mycotoxins in this region can have a devastating human effect **(Bryden, 2007).** In developing countries it is likely that consumers will be confronted with a diet that contains a low level of toxin and in many cases there may be other toxins present **(Bryden *et al*., 2001).** Suppression of cellular immune system is a known result after ingestion of several mycotoxins **Stoev *et al*., (2000).**

During the dry season (period of experiementation), ambient temperature is high and the dry paddock environment is predisposed to dust (fungi mycoflora are easily dispersed through dust), hence snails could be contaminated by air containing fungal spores. Inhalation is a route of toxic bioaccumulation **(Ebenso and Ologhobo, 2008).** The potential health hazards of mycoflora need not be overemphasized, because, fungi following inhalation are capable of surviving in the respiratory and intestinal tract, where they may continue to grow and produce their toxins **(Oyero and Oyefolu, 2011; Frisvad *et al*., 2007).** According to **Ebenso and Ebenso, (2011)** children who eat more than 0.18kg/meat of snail meat, and above African maximum permissible limit for aflatoxin of $20mg^{-kg}$ **(Oyero and Oyefolu, 2011)** will be at risk of additional chance of developing adverse health conditions. Reports of **Hendrickse, (1991)** linked kwashiorkor to aflatoxin exposure.

A big setback is that most mycotoxins are heat resistant within the range of conventional food processing temperature (80-120^{o}C) of normal cooking conditions such as boiling and frying or even following pasteurization **(Zaki, 2012)** every effort to prevent mycotoxins should be integrative of physical, chemical and biological systems.

It can be speculated from the present study that livestock that grazed the paddocks could present animal products of inferior market quality that could attract discount prices (such as meat, animal skin and hide), could be carriers of sub-clinical mycotoxic illnesses, and human population could are at risk of adverse health effects after consumption.

Table 1 The pathogenic profile (cfu^{-g}) isolated from proximal gut of A. *marginata* at grazing paddocks

Sampling location	Total bacteria	Mycotoxins	*Salmonella*	*E. coli*
Old paddock (abandoned)	$2.5x10^{7\ a}$	$3.0x10^{6\ a}$	$0.00^{\ b}$	0.00
New paddock (currently in use)	$2.6x10^{7\ a}$	$2.0x10^{6\ b}$	$0.00^{\ b}$	0.00
Control (reference site)	$1.55x10^{8\ b}$	$1.2x10^{6\ c}$	$3.00x10^{6\ a}$	0.00

Legend: abc means with different alphabets in a column are significantly different by Duncan Multiple Range Test $\alpha = 0.05$

Table 2 Positive samples (n=100) of microscopic fungal isolates ($cfu^{-g}x\ 10^5$) from proximal gut of *A. marginata* at grazing paddocks

Fungi	Control (reference site)	Old paddock (abandoned)	New paddock (currently in use)
Aspergillus niger	4	7	2
Phoma lingam	0	0	5
Rhizopus stolonifer	2	5	2
Cladosporium fulvum	1	2	0
Monilia sitophila	0	5	0
Penicillium expansum	3	0	9
Mucor mucedo	1	0	2
Aspergillus fumigatus	1	7	0
Humicola fuscoatra	0	4	0

Table 3 Morphological examination and names of fungal isolates from proximal gut of *A. marginata* at grazing paddocks

Isolate code	Colony colour	Types of soma	Nature of hyphae	Special vegetative structure	Asexual spore	Special reproductive structure	Conidial head	Vesicle shape	Probable organisms
1	Yellow basal dark colony	Filamentous	Septate	Footcell	Globose conidia	Smooth walled, erect conidiophores	globose	Globuse	*Aspergillus niger*
2	White with blackish brown	Filamentous	Septate	-	Chlamydospores 1-cell ovoid conidia	Pyenidia	-	-	*Phoma lingam*
3	White grayish brown	Filamentous	Coenocytic	Stolons, Rhizoids	Ovoid conidia	Tall, sporagiophores in groups, black brown	-	-	*Rhizopus stolonifer*
4	Powdery Olivaceous brown	Filamentous	Septate	-	Acropetal branches	Short conidiophores	-	-	*Cladosporium fulvum*
5	White	Filamentous	Septate	-	1-cell conidia in chains	Conidiophores hypae	-	-	*Monilia sitophila*
6	Blue-green colony	Filamentous	Septate	Broom-like appearance	Sub-globuse conidia	Highly 3-stage branched conidiospores	-	-	*Penicillum expansum*
7	Greenish yellow	Filamentous	Coenocytic	-	Sporongiospore	Sympodially branched sporangiophore, zygospore	-	-	*Mucor mucedo*
8	Grey-green colony	Filamentous	Septate	Footcell	Globose conidia	Short conidiophores	Typically columnar	Dark shaped broadly clavate	*Aspergillus fumigatus*
9	Dark	Filamentous	Septate	-	Aleuriospore phialospores in chains	Erect conidiophores with short branches	-	-	*Humicola fuscoatra*

CONCLUSION

Aspergillus and *Penicillum* were detected as potential toxicogenic mycoflora in the study of epidemiology of livestock environment. These foodborne mycotoxins are food safety risk which could pose adverse health effects to both human and livestock as moulds frequently occur in animal feed ingredients and related farm commodities.

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