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Acute Toxicity of Mycotoxigenic Fungi Isolates in Ready-to-Eat Meat in Buea, Cameroon

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Abstract:

Introduction: Meat is an important source of nutrition for many people worldwide. Away from homes, it is widely consumed in restaurants (e.g. small roadside) and non-restaurants (including drinking parlors and open-air)in various forms assnack. *Objective*: This paper reports on the acute toxicity of mycotoxigenic fungi isolates from various ready-to-eat meat from Buea, Cameroon.

Method: Ten varieties of ready-to-eat meat samples (N=130: raw beef (n=18), beef soya (n=29), fried chicken (n=11), chicken soya (n=10), snail soya (n=13), fried pork (n=06), fried cow liver (n=13), roasted goat (n=06), boiled cow skin (n=07) and smoked beef (n=17).were collected from Buea municipality. The samples were cultures, and fungal isolates isolated microscopically. Thereafter, an acute toxicity test using 24 young BALB/c mice (weight range: 22-25g) was performed.

Results: A total of 40 mycotoxigenic fungi belonging to five genera (*Aspergillus, Penicillium, Fusarium, Rhizomucor,* and *Rhizopus*) were identified. The most dominant species was *Aspergillus* (unidentified *Aspergillus* species, 23.1%; and *Aspergillus ochraceus,*10.3%).The order of *Aspergillus* fungi proliferation were roasted goat liver(20%), fried chicken (20%), snails soya (20%), Fried Cow liver(13.3), raw beef (13.3%), chicken soya (13.3%), smoked beef (6.7%), boiled canda (6.7%) and beef soya(6.7%). The mean body weight of test mice significantly decreased ($p<0.05$) after the first 7 days of feeding with a mycotoxigenic fungi-supplemented diet relative to control mice that ate healthy diets. Aspartate Transaminase (AST, range: 169.9-563.1U/L) and Alanine Transaminase (ALT, range:521.1-707.5U/L) levels were significantly ($p<0.05$) higher than the reference values for healthy mice (range: 60-100U/L for AST and 25-6U/L for ALT).

Conclusion: Given the toxicity potentials of mycotoxigenic fungi, e.g. production of toxic secondary metabolites, not reported in this paper, and demonstrated by the liver toxicity/function test, ready-to-eat meat in Buea, Cameroon may not be adequately safe for consumption.

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1. INTRODUCTION

Meat is an important source of nutrition for many people worldwide. Demand for meat has quadrupled globally in the last 50 years, with an estimated 320 million tons of meat produced annually from approximately 80 billion animals [1]. In Cameroon, like elsewhere in sub-Saharan Africa, protein-energy undernutrition (PEU) (formally protein-energy malnutrition, is a public health issue and remains a vital risk factor for several diseases and death across all age groups [2-5]. PEU may manifest in several forms including kwashiorkor and marasmus all provoked partly due to deficiency in macronutrients (protein, carbohydrates, and fat) [3,6]. In Cameroon, calorie-driven staple plant-based foods such as cassava-based diets are more readily available and accessible to the masses relative to animal-based protein-rich foods like meat; a source of complete protein. This may be due to the economic unaffordability of meat by the masses [7]. Worse still, ready-to-eat meat are easily exposed to contaminants [8,9] especially when sold asa snack.

Contamination of meat is partly influenced by its rich nutritional contents that favours the growth of microbes such as fungi, which are ubiquitous by nature and cause food spoilage [10]. Meat (and meat products) can be contaminated by toxigenic fungi species, primarily during processing and storage. The main genera involved are *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* [11,12]. Contamination can occur through direct contact with molds or indirectly via contaminated animal feed [11]. The presence of mycotoxigenic fungi species in meat products may be suspicious of health risks when considering their potential to produce mycotoxins which are potentially carcinogenic, mutagenic, immunotoxic, and teratogenic. Approximately, 1.5 million species of fungi are in existence [13], but only about 70,000 species have so far been described [14,15]. Fungi easily contaminate foodstuffs and produce their toxic secondary metabolites, mycotoxins, at temperatures above 30°C and relative humidity over 80%, both in the field and during storage [16. Additionally, conditions such as nutrient availability [17], O_2 supply [18], and water activity ($_a$w) are equally important for fungal growth. Furthermore, [19] considered different factors, including soil infertility, poor crop production management, disease prevalence, or insect infestations to be responsible for increased microbial contamination in the field and during storage. Due to poor sanitation and handling procedures, food stuffs are prone to mycotoxin contamination (from fungal growth) along the food supply network. For example, feeding animals with mycotoxins-contaminated feeds is the main source of mycotoxin contamination of farm animals [20] and by extension man, the end-product consumer of the animals as meat. Human exposure to mycotoxins is mainly through consumption of mycotoxin-contaminated foods (dietary exposure) [21]. Altogether, these toxins serve as risk factors for several human diseases and constitute a public health menace whether through chronic or acute exposures [21-26].

Extensive work have been done regarding food contamination by bacteria but that is not the case with fungal even though they have may adverse health effects. The reports about fungi are mostly on agricultural products like maize and cassava but none has been done on ready to eat meat in Cameroon. Considering the preliminary toxigenic fungi reports from Cameroon, a total of 13 *Aspergillus* species were isolated with *A. clavatus, A. flavus, A niger, A*. *fumigatus*, and *A*. *ochraceous* constituting over 70 % of total isolates from 72 cassava chips [27]. Similarly, *Aspergillus* strains (dominated by *A. flavus* and *A. parasiticus*), *Penicillium* (mainly *P. polonicum* and *P. crustosum*) as well as *Fusarium* were identified with 85% incidence of co-contamination of *Aspergillus* and *Penicillium spp*. in human food samples with over 50 % of the fungal isolates having mycotoxigenic potentials [28].The existing dataset focuses on plant-based foods with none on animal-based food. However, most ready-to-eat meat are sold/served exposed in various forms such as soya/suya, and roasted chicken, pork, or goat meat not leaving out fried snail meat, making it more susceptible to contamination even before it reaches the consumers. There is limited information on fungi/mold contamination in ready-to-eat meat in Cameroon. This study aimed to identify mycotoxigenic fungi species *in ready- to- eat meat types and to assess* acute toxicity of the fungi from selected meat types using albino mice.

2. MATERIALS AND METHODS

2.1. Study Site, Target Population, and Sampling

Samples of ready-to-eat meat were purchased from roadside vendors, butcher's shops, restaurants, and eateries in the Bue Municipality. The samples (N=130) were raw beef (n=18), beef soya (n=29), fried chicken (n=11), chicken soya (n=10), snail soya (n=13), fried pork (n=06), fried cow liver (n=13), roasted goat

Table 1: Selected Samples for Acute Toxicity Test and their Groups

Groups:	G1	T1	T2	T3	T4	T5
Mold samples:	Control	*Aflatoxin flavus*	*Penicillum expansum*	*Aflatoxin ochraceus*	*Aflatoxin niger*	*Fusarium oxysporum*

(n=06), boiled cow skin (n=07) and smoked beef (n=17). The samples were placed in plastic bags and transported to the laboratory. Each Sample was dried at 35°C for two days and milled into powder using a hand machine (CAM2 304 Stainless Steel Heavy Duty Manual Meat Grinder) The milled samples were then placed in zip-lock plastic bags, and preserved at -20°Cuntil the analysis for presence of mycotoxigenic fungi was done.

2.2. Fungal Isolation

2.2.1. Culturing and Isolation

Potato Dextrose Agar (PDA) used for culturing was purchased from BIOPHARCAM Sarl, Buea (manufactured by Jinan BaiboBiotechnology Co., LTD: Jinan City, Shandong Province of China). The culturing was done according to the manufacturer's instructions. For initial isolation, inoculation was done by streaking the samples on the plates using an inoculation loop under a hood near a flame to prevent any form of contamination. The plates were sealed with paraffin foil paper to reduce contamination by the incubator (Heraeus 27004776, Germany), set at 25°Cfor 5 days, after which the plates were checked and well-isolated colonies were picked up and transferred to new PDA. The operation was repeated twice to ensure purification. This gave a picture of the total fungi present in each studied meat sample. All the sub-cultures on the PDA were labeled to distinguish each fungus to have clear identification. Macroscopic identification was appreciated after the fifth day by observing the front and inverse views of cultured plates. Fungi were identified based on the morphological and cultural characteristics. The colour, morphology, and hyphae of the colonies were taken note of to give the microscopic characteristics of the fungi according to the method of [29].

2.2.2. Microscopic Identification of Isolates

A wet mount slide was made by transferring a small amount of the culture with a sharp dissecting needle to a drop of physiological saline on a grease-free glass slide (NANJING EVERICK, China). A drop of lactophenol cotton blue stain was then added to each slide. A cover glass (ASSISTANT, Germany) was then affixed on the wet mount and examined under a microscope (HumanScope^{PLUS}, Germany) using the low power (X40) objectives. Hyphae and spore morphology were snapped to record the microscopic presentation of fungi.

2.3. Selection of Mice for Acute Toxicity Studies

Twenty-four (24) young BALB/c mice (mean weight: 24.04 ± 2.8g; age: 3 weeks old) were obtained from the University of Buea, Faculty of Science Institutional Animal Care Committee (IACUC). They were placed into 6 groups (test groups 1-5 and control group) of 4 mice each and each group was housed in a plastic cage. Their housing conditions were kept at 25°C with a 12-hourlight/ dark cycle and were fed normal diets for a one-week acclimatization period. Weights of mice were used to calculate the dosages of extracts each animal received.

2.4. Sample Processing for Acute Toxicity Test Using Enzyme Bioassay

Only the meat with potent mycotoxigenic fungi isolates identified macroscopically and microscopically from the cultured samples were considered for acute toxicity studies. Meat extracts were prepared by dissolving the calculated amount of the dried powdered meat samples into the premeditated volume of distilled water in a beaker. The extracts in the beaker were thoroughly stirred using a vortex (electronic stirrer) so that they dissolved properly. The mice were given single oral limit doses of the different meat extracts using a gavage needle while control mice received extracts made from uncontaminated meat samples from a mycotoxigenic fungi species perspective. The treated mice were monitored for signs of toxicity and mortality at the first, second, fourth, and sixth hours for mortality within 24 hours. They were further observed daily for an additional 14 days, for signs of delayed toxicity. The body weight of the studied mice was recorded before the treatment period, on the7th and 14th day. The mean body weights for each group were also calculated. All surviving mice in all the groups were fasted for 24 hours and sacrificed for necropsy examination. Before sacrifice, anesthesia was induced by administration of 1% (v/v) chlorose in

25% (v/v) urethane (w/v) (5mL/kg). Blood was collected from the heart for hematological and biochemical analysis. The blood was centrifuged at 2000rpm/15mins to obtain dark red colored sera. Figure **1** shows the schematic presentation of the toxicity studies.

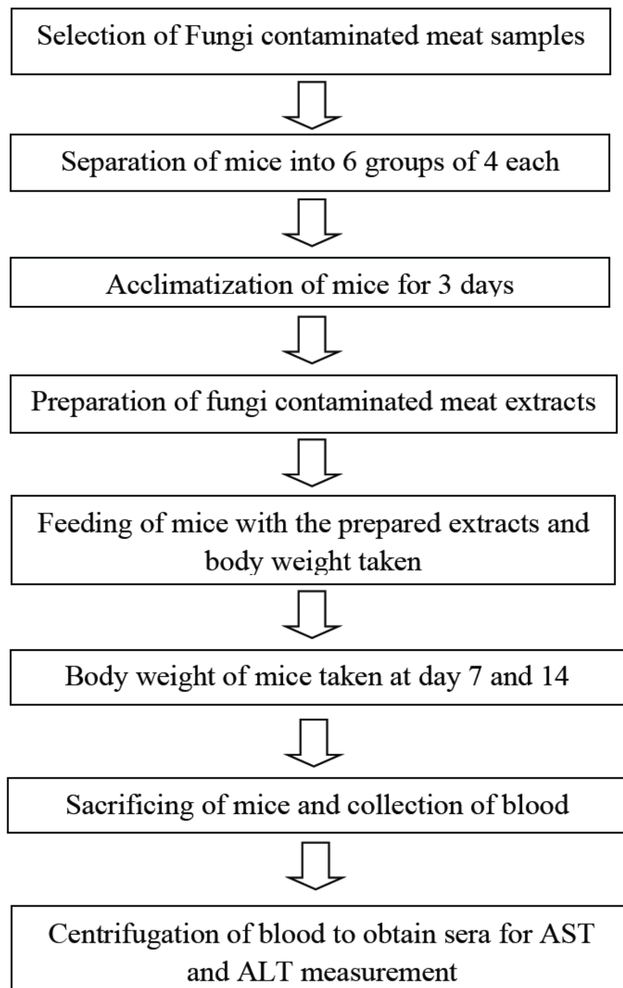

Figure 1: Schematic presentation of toxicity study protocol.

2.5. Measurement of Liver Function Enzymes (Alanine Transaminase, ALT, and Aspartate Transaminase, AST)

The test kits measuring Alanine Transaminase (ALT) and Aspartate Transaminase (AST) levels in serum were purchased from BIOPHARCAM Sarl, Buea (manufactured by SPECTRUM and BIOSYSTEMS). Reagents were mixed according to the manufacturer's instructions and quantified through spectrophotometry. Preparation of the detection reagent was done according to the manufacturer's instructions by mixing 5mLof R1 (80mmol/L of TRIS and 200mmol/L of L-Aspartate) buffer and 1ml of R2 (0.18mmol/L of NADH, 800U/L of Lactate dehydrogenase, 600U/L of Malate dehydrogenase and 12mmol/L of α - ketoglutarate) substrate. Thereafter, 10µLof each serumsample was pipetted into acuvette. 40µLof normal saline was added to it followed by 300µLof detection reagent. The content in the cuvette was properly mixed by shaking and the absorbance read at 340nm. The calibration was done using normal saline as the blank to get the initial absorbance before starting the stopwatch to get other absorbance from the spectrophotometry readings. The above reactions were carried out at 37°C in a water bath and measured at 340nm. The concentration of ALT and AST in sera is expressed in U/L

Change in absorbance per minute x 1750 = U/L of ALT/AST.

ΔA/min x 1750 = U/L of AST

Where: "1750" is the extinction coefficient and "A" is absorbance.

2.6. Statistical Analysis

Microsoft Excel 2010 was used to enter the data, calculate mean, standard deviation, and plotcharts. The Statistical Package for Social Sciences(SPSS) version 20.0 was used to carry out Pearson correlation and a two-tailed test at a significance level of $p<0.05$ to check the correlation of AST and ALT in the various groups of animals.

3. RESULTS

3.1. Characterisation of Presumptive Fungi

There was visible fungal growth in 34 of the 50 cultured plates. 10 of the 34 cultured plates had more than one type of mold growing in the plate while 24 had just one type of fungal growth. The different mold isolates were identified using their cultural characteristics (macroscopic observation) and microscopically after staining with lactophenol cotton blue stain.

Table **3** below shows the different identified fungi based on morphological and microscopic characteristics. The molds isolated showed the molds isolated showed characteristics macroscopic and microscopic differences making identification of these molds to the genus level and for some, to the species possible'.

The combination of the morphological (i.e., aspects of a mold that can be appreciated by the naked eyes

Table 2: The Number of Visible Fungi Species According to Sample Types

Sample type	Location	Number of species isolated
Smoked beef	B/da Foncha	01
Snails soya	Muyuka	04
Beef soya	Muyuka	02
Goat soya	Muyuka	01
Beef soya	Buea	07
Chicken soya	Buea	03
Fried cow liver	Buea	04
Fried cow lungs	Buea	01
Fried goat liver	Buea	07
Snails soya	Buea	03
Raw beef	Buea	05
Pepper soup Pork	Buea	01
Fried chicken	Buea	06
Fried canda	Buea	02

such as color and texture) and microscopic (i.e., the reproductive features such as hyphae, spores, and mycelium that can only be seen clearly under the microscope) characteristics led to easy identification of the given mold specie.

Table **4** shows that snails and raw beef had the most contamination frequency (15.2%). Ten of the 34 samples that had mold growth were snails and raw beef (5 each). Smoked beef, cow liver, and beef soya had the second most positives (12.1%). This was followed by fried chicken and goat liver (9.1%). Roasted chicken and boiled canda (cow skin) had 6.1% positives and finally pork with 3%.

3.2. Macroscopic and Microscopic Views of Proliferated Colonies

The various mold isolates showed macroscopic and microscopic differences in their characteristics making it possible to identify some genera and their species. Isolates belonging to the genus Aspergillus were dominant with 10 different species identified including *Aspergillus flavus, A. sulphureus, A. ochraceus, A. niger, A. candidus,* and five other non-identified species coded *Aspergillus sppa, Aspergillus sppb, Aspergillus sppc, Aspergillus sppd* and *Aspergillus sppe.*

The next dominant isolates belonged to the genus *Penicillum*, with 3 different species identified including *P. expansum*, *P. chrysogeneum, P. corylophilum*. The next isolates belonged to the genus *Fusarium* with *oxysporum*as the only identified species followed by two unidentified species “a” and “b”. The next identified isolates were single species each from the general *Rhizopus* and *Rhizomucor*

Figures **2** to **5** show the different fungi species, their microscopic structure, the macroscopic picture of fungi in the medium, and all the samples with the same fungi species.

3.3. Toxicity Findings

3.3.1. Correlation of Animal’s Body Weight

Figure **6** shows the mean body weights in the various sub-groups of test mice. Mean body weights decreased from day 1 to day 7 significantly ($p<0.05$) in all test groups while the control group had an increase but not statistically significant ($p>0.05$). The increase in body weight from day 7 to day 14 in all test groups was significant ($p<0.05$) but not for the control group.

3.3.2. Liver Function Enzymes [Aspartate Transaminase (AST) and Alanine Transaminase (ALT)]

The concentrations of the liver function enzymes (AST and ALT) are presented on Figure **7**. The highest enzyme amounts were observed in the sub-groups fed *F. oxysporum* (AST) and *A. flavus* (ALT), both seconded by *A. niger*. Meanwhile, AST and ALT levels in *A. niger* and *P. expansum* diet-supplemented sub-groups were respectively higher relative to their

Table 3: Colonial Characteristics of Isolates from Meat

Meat Types	Morphological Characteristics		Microscopic Characteristic	Mold Species
	Surface View	Reverse View		
Fried snails	Powder grey colonies	Pale yellow to brown	Aseptate conidiophore	*Aspergillus ochraceus*
	Floccose bright yellowish-green colonies	Pale yellow to brown	Aseptate conidiophores Globular conidia	*Aspergillus spp* a
	Velvet-like yellowish brown with white margins	Yellow to cream white	Aseptate conidiophore	*Aspergillus spp* b
	Velvet–like dark green with clear white margins	yellow	Velutinous conidiophore	*Penicillum expansum*
	Elevated white cotton-like colonies overlying mucous layer	Colorless	Sporangia having spores. Rhizoids present	*Rhizomucor*
Roasted goat liver	Velvet-like black colonies	Cream white to pale yellow	Aseptate conidiophores Small dark conidia	*Aspergillus niger*
	Powder grey colonies	Pale yellow to brown	Aseptate conidiophore	*Aspergillus ochraceus*
	Velvet-like yellowish brown with white margins	Yellow to cream white	Aseptate conidiophore	*Aspergillus spp* b
	Velvet-like – blue-green to grey-green colonies.	Green to dark green colonies.	Smooth layer of conidiosphore	*Penicillium corylophilum*
	Floccose Magenta pink	Magenta to violet	Three – septate spores	*Fusarium oxysporum*
	Elevated white cotton-like colonies overlying the mucous layer	Colorless	Sporangia having spores. Rhizoids present	*Rhizomucor*
Fried cow liver	Grey colonies	Pale yellow to brown	Aseptate conidiophore	*Aspergillus ochraceus*
	Elevated yellowish brown with a white margin	Pale yellow to cream white	Aseptate conidiophore	*Aspergillus spp* b
	Velvet–like bright green with yellow margin	Cream white	Brush-shaped conidiopshores	*Penicillium chrysogeneum*
	Velvet–like yellowish brown with white margins	Yellowish to cream white	Three – septate spores	*Fusariumspp* a
	Elevated white cotton like colonies overlying mucous layer	Colorless	Sporangia having spores Rhizoids present	*Rhizomucor spp*
Beef soya	Velvet – like yellow to brown with dark spores at the centre	yellow	Septatehypae having mycelia	*Aspergillus flavus*
	Velvet –like bright green with clear white margins	Creamish white	Brush-shaped conidiopshores	*Penicillum chrysogeneum*
	Velvet-like dark green with a sterile white margin	yellow	Velutinous conidiophore	*Penicillumexpansum*
	Floccose Magenta pink	Magenta red to violet	Three – septate spores	*Fusariumoxysporum*
	Elevated white cotton-like colonies overlying mucous layer	colorless	Sporangia having spores. Rhizoids present	*Rhizomucorspp*
Fried chicken	Velvet- like cream white colonies	Slightly creamish	Septate branched hyphae with conidial heads.	*Aspergillus candidus*
	Elevated pale yellow	Cream white	Septate conidiosphore	*Aspergillus spp* c
	Velvet – like yellowish green	Pale yellowish	Septate and smooth conidiophore	*Aspergillus spp* d
	Velvet – like dark green	Cream white to yellowish	Velutinous conidiophore	*Penicillum expansum*
	Floccose pink	Magenta red	Pigmented with three–septate spores	*Fusarumoxysporum*

(Table 3). Continued.

Raw beef	Flat yellowish green	Yellow	Flat yellow filaments (hyphae)	*Aspergillus spp* c
	Velvet–like pale yellow colonies	Grey to light green	Smooth conidiophores and spores.	*Aspergillus spp* e
	Elevated white cotton-like colonies	Colorless	Sporangia having spores. Rhizoids present	*Rhizomucorspp*
	Elevated white cotton like growth with black spots	colorless	Sporangia having spores. Umbrella-like columellae	*Rhizopus spp*
Smoked beef	Velvet–like yellowish brown with white margins	Yellow to cream white	Aseptate conidiophore	*Aspergillusspp* b
	Velvet – like dark green	yellow	Velutinous conidiophore	*Penicillium expansum*
	Flat white cotton-like colonies	Colorless	Sporangia having spores. Rhizoids present	*Rhizomucor*
Boiled cow skin (canda)	Velvet-like yellowish brown with white margins	Yellow to cream white	Aseptate conidiophore	*Aspergillus spp* b
	Floccose pink	Reddish	No pigment with three-septate spores	*Fusariumspp* b
Fried pork	Elevated white cotton-like colonies	Colorless	Sporangia having spores. Rhizoids present	*Rhizomucor*
Roasted Chicken	Grey colonies	Pale to grey colonies	Aseptate conidiophores	*Aspergillus ochraceus*
	Velvet-like pale yellow colonies	Greytolightgreen	Smooth conidiophores and spores	*Aspergillus spp* e
	Elevated white cotton-like growth with black spots	Colorless	Sporangia having spores	*Rhizopus spp*

Table 4: Frequency of Fungi Contamination of Meat Samples

S No.	Meat Sample (total Number, N=130)	No. of Positive Samples (%)	Number of Detected Fungi Species	
			Number	Fungi
1	Snails soya (n=13)	5 (15.2)	5	*Aspergillus ochraceus, Aspergillus spp* a, *Aspergillus spp* b, *Penicillum expansum, Rhizomucor.*
2	Raw beef (n=18)	5 (15.2)	4	*Aspergillus spp* c, *Aspergillus spp* e, *Rhizomucor, Rhizopus*
3	Beef soya (n=29)	4 (12.1)	5	*Aspergillus flavus, Penicillum chrysogeneum, Penicillum expansum, Fusarium oxysporum, Rhizomucor*
4	Roasted goat liver (n=17)	4 (12.1)	6	*Aspergillus niger, Aspergillus ochraceus, Aspergillus spp* b, *Penicillium corylophilum, Fusarium oxysporum, Rhizomucor*
5	Fried cow liver (n=13)	4 (12.1)	5	*Aspergillus ochraceus, Aspergillus spp* b, *Penicillium chrysogeneum, Fusarium sppa, Rhizomucor*
6	Fried chicken (n=11)	3 (9.1)	5	*Aspergillus candidus, Aspergillus spp* c, *Aspergillus spp* d, *Penicillum expansum, Fusarium oxysporum*
7	Smoked beef (n= 06)	2 (6.1)	3	*Aspergillusspp* b, *Penicillium expansum, Rhizomucor*
8	Chicken(roasted) soya (n=10)	2 (6.1)	3	*Aspergillus ochraceus, Aspergillus spp* e, *Rhizopus*
9	Boiled cow skin (canda) (n=07)	2 (6.1)	2	*Aspergillus spp* b, *Fusarium spp* b
10	Fried pork (n=06)	1 (3)	1	*Rhizomucor spp*
Total	*10*	*32 (sum of % /32)*	*Total no of species in all* N=39	

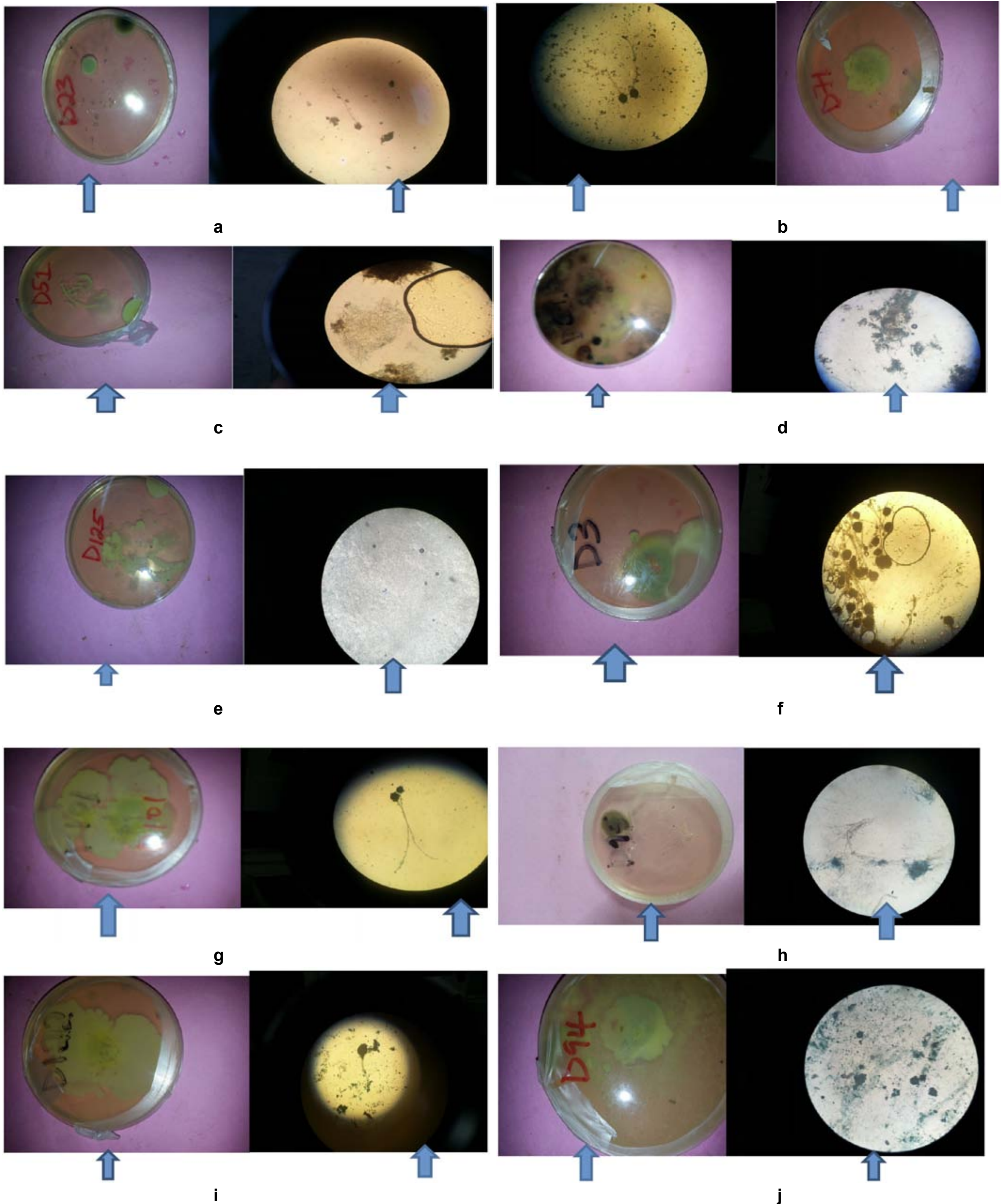

Figure 2: a: Inverted view (yellow to brown with dark spores at the centre), view under a light microscope (*Aspergillus flavus*) respectively. **b:** Inverted view (dark colonies), view under a light microscope (*Aspergillus niger*) respectively. **c:** Inverted view (Dirty white with yellow spores at the centre, view of under light microscope (*Aspergillus sulphureus*) respectively. **d:** inverted view (grey colonies), view under light microscope (*Aspergillus ochraceus*). **e:** Inverted view (slightly creamish), view under a light microscope (*Aspergillus candidus*). **f:** Inverted view (bright yellowish green), view under a light microscope (*Aspergillus spa*) respectively. **g:** Inverted view(yellowish brown with white margin), view under light microscope (*Aspergillus spp* b) respectively. **h:** inverted view (yellowish brown), view of slide under a light microscope (*Aspergillus spp* c). **i:** Inverted view (pale yellow), view under light microscope (*Aspergillus spp* d). **j:** Inverted (Grey to light green), view under **a** light microscope (*Aspergillus spp* e).

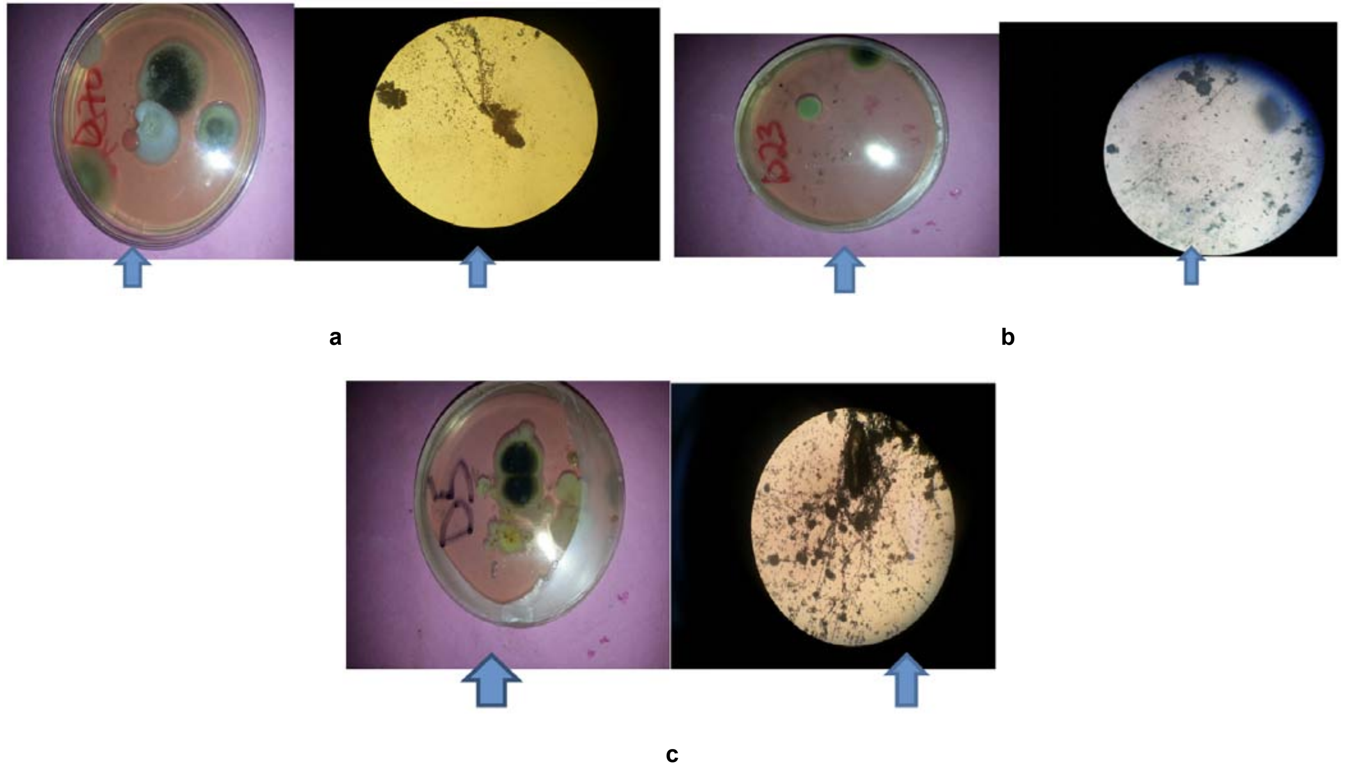

Figure 3: a: Inverted view (magenta pink), view under a light microscope (*Fusarium oxysporum*). **b:** Inverted view (pink), view under light microscope (*Fusarium spp a*) respectively. **c:** Inverted view of plate (white colonies with pink center), view under a light microscope (*Fusariumspp* b) **e:** Inverted view (white cotton-like colonies), view under light microscope (*Rhizomucor spp*).

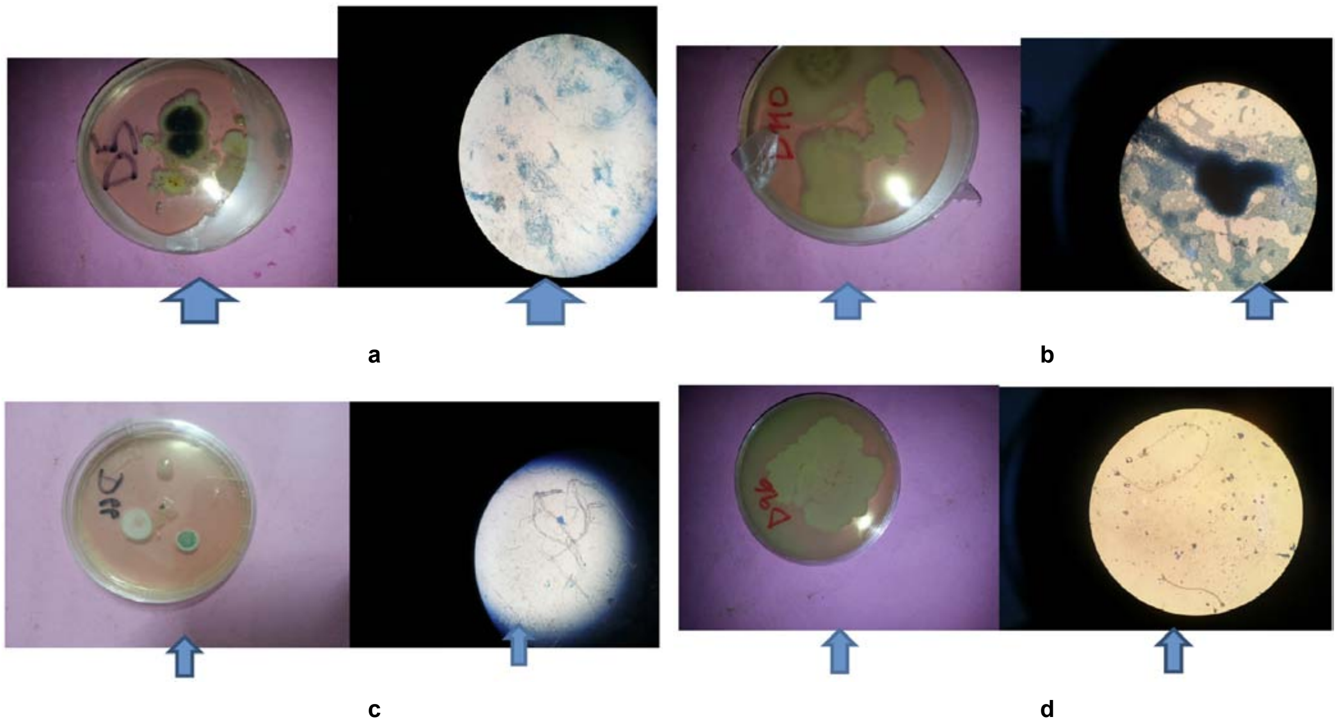

Figure 4: a: Inverted view (magenta pink), view under a light microscope (*Fusarium oxysporum*) **b:** Inverted view (pink), view under light microscope (*Fusarium spp* a) respectively. **c:** Inverted view of plate (white colonies with pink center), view under a light microscope (*Fusarium spp* b). **d:** Inverted view (white cotton-like colonies), view under light microscope (*Rhizomucorspp*).

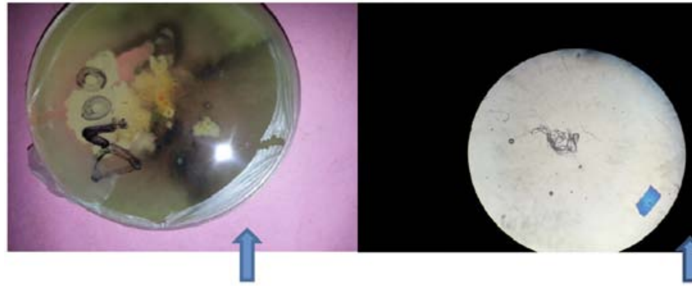

Figure 5: Inverted view (white cotton-like growth with black spores), view under light microscope (*Rhizopus spp*).

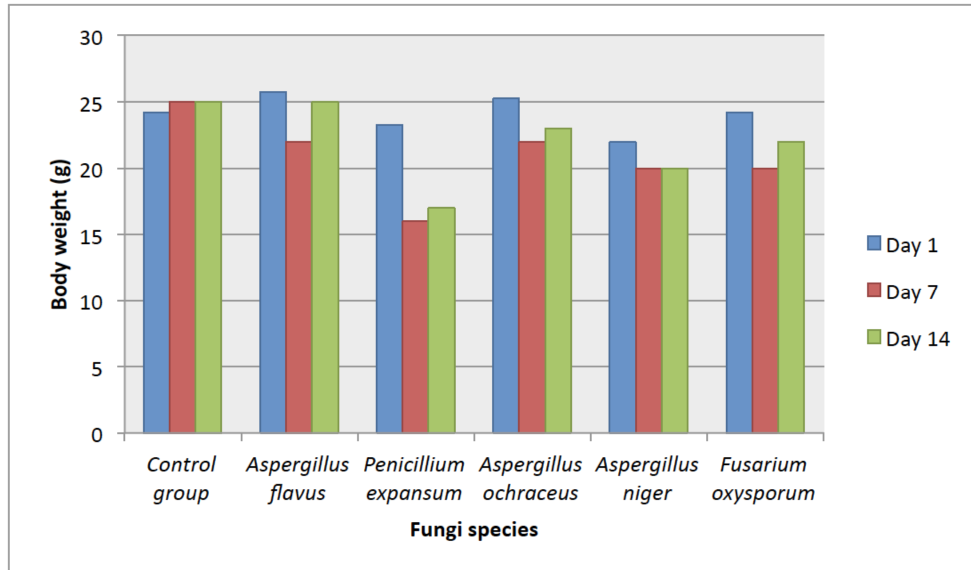

Figure 6: Relationship of mean body weights of studied mice for days 1, 7 and 14.

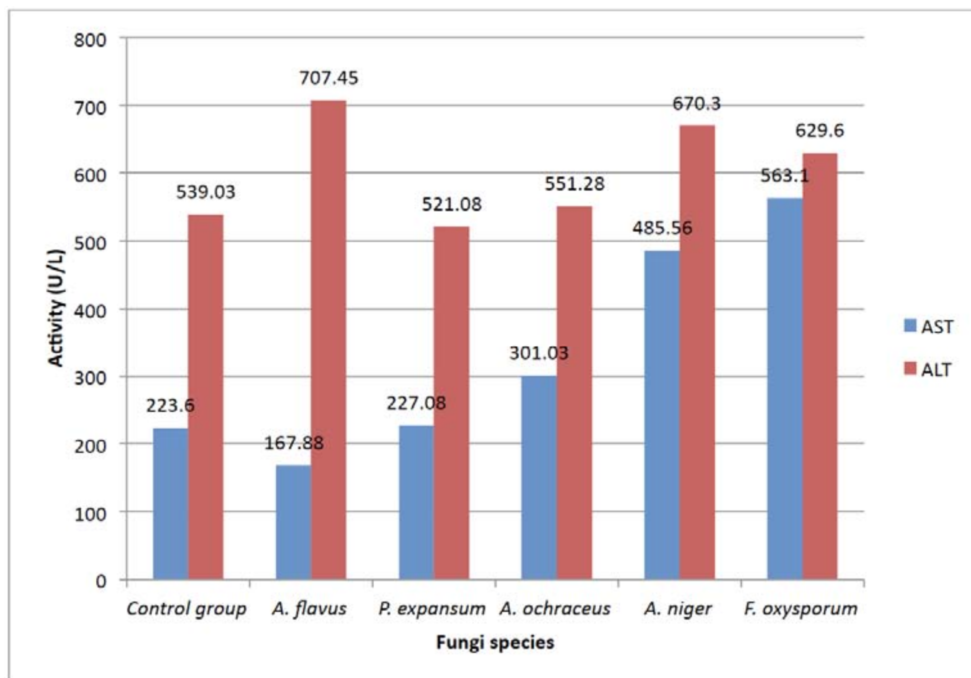

Figure 7: Relationship of Mean AST and ALT.

individual control group level. All studied liver function enzyme activities were significantly ($p<0.05$) elevated in all sub-groups; test and control alike.

4. DISCUSSION

The study was conducted in Muyuka, Muea and Buea areas located within the South-West Region of Cameroon, which have an equatorial climate. It rains almost throughout the year. Annual rainfall often averages 1500mm [30]. This rainfall implies high humidity which promotes the growth of many microorganisms that can influence the final quality of meat and meat products consumed in the region. Molds and their toxins have been established as important food-poisoning organisms. This has been partly attributed to their wide distribution in nature as their presence in food products can be traced back to the environment [31]. The ubiquity of these organisms makes it quite difficult to prevent their contamination of food and food products. Except for a few unidentified *Aspergillus* species, all the molds isolated in this work have been previously reported to cause spoilage of food and have been isolated from various food items such as cotton seeds [32], cassava [33].

Aspergillus were the dominant genera identified in this work similar to the findings of [34] but the species isolated were not the same. The species of *Aspergillus* isolated in this work were as follows; *Aspergillus flavus, Aspergillus ochraceus, Aspergillus niger, Aspergillus candidus, Aspergillus sulphurous* and five other unidentified species, while [34] isolated *Aspergillus niger, Aspergillus flavus, Aspergillus parasiticus,*and *Aspergillus versicolor.* These findings are partly similar to that of [35,36] who isolated some of these *A spergilli* from luncheon in Egypt and fermented meat products in Slovakia.

Generally, mold growth may introduce a meat product to consumers containing aflatoxins, ochratoxin, and other mold metabolites like antibiotics and allergens, which pose health dangers to consumers.

The findings of this work partly correspond with those of [37], whoreported that the isolated fungi genera from retailed meats are *Aspergillus, Penicillium, Alternaria, Cladosporium, Mucor,* and *Rhizopus.* Additionally, [38], noted that *Penicillium* and *Aspergillus* were the main isolated moulds from sausage marketed in Italy. Mold contamination of meat samples observed in this study may indicate inadequate sanitary measures performed during the processing of such products leading to an increase in the initial microbial load. Additionally, inadequate storage conditions like fluctuation in freezing temperature may lead to high mold contamination of such products since some of the meat may have undergone such storage, for example, sharwama. The conditions of the environment in the manufacturing rooms, stores, refrigerators, and shops are suitable for the development of molds inside the products, but more frequently on the surface of various sorts of meat and meat products [36].

The result of this study is in contrast with that of [39] who reported that the administration of *P. resticulosum* pigment for 28 days produced no effect on the body weight gain and abnormalities in the liver and kidneys of mice. The mean body weight changes observed in this study show that the oxygenic molds in the meat extracts may probably have caused the weight loss.

In this study, the observation for liver enzymes is contrary to that of [39] who showed that *P. resticulosum* pigments up to a dose of 500mg/kg body weight daily for 28 days had no effect on AST and ALT activities. The significant ($p<0.05$) elevated values of AST and ALT concentration in sera could be an indication of liver and kidney damage. Liver and heart injuries may be due to the fact that poly-molds contaminated meat extracts were given to the mice. Injuries to the liver and kidney can cause a massive leakage of these liver enzymes in blood. Tissue activities of the transaminase (AST and ALT) enzymes are markers for the functions and integrity of the heart and liver [40].

5. CONCLUSION

Five genera with different mold species were isolated from different ready-to-eat meat samples. Snails and beef soya were more contaminated than pork and fried chicken. Toxigenic molds caused a significant ($p<0.05$) reduction in mice body weights within 7 days of oral dose administration of meat extracts. Mean ALT concentrations were significantly ($p<0.05$) higher than that of AST and finally all mean AST and ALT concentrations in groups fed with contaminated meat extracts and the control group were higher than the reference values in normal mice. Meat is good but we should be careful when consuming them because they are susceptible to contamination by mycotoxins which causes adverse effects to our health.

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