LEVELS OF AFLATOXINS IN FLOUR INGREDIENTS FROM MICRO, SMALL AND MEDIUM ENTERPRISES IN NAIROBI COUNTY AND THE EFFECT ON FEEDING RATS

BY

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To God for whom nothing is impossible,

The powerful intercession of Blessed Don Alvaro,

My loving parents for your unfailing affection concern and support.

Words are too short to express my utter gratitude.

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ABBREVIATIONS/ACRONYMS

AfB_1/B_1	Aflatoxin B_1
AUC	African Union Commission
B_2	Aflatoxin B_2
CIYMMT	International Maize and Wheat Improvement Centre
FAO	World Food Organization
FSNT Technology	Department of Food Science, Nutrition and
G_1	Aflatoxin G_1
G_2	Aflatoxin G_2
GEE	Generalized Estimating Equations
HCC	Hepatocellular Carcinoma
IARC	International Agency for Research on Cancer
KARI	Kenya Agricultural Research Institute
LD_{50}	Lethal Dose at 50%
MSME	Micro Small and Medium Enterprise
OD	Optical Density
PACA	Partnership for Aflatoxin Control in Africa
PPB	Parts Per Billion
PHPT	Department of Public Health, Pharmacology and Toxicology
REWGA	Regional Experts Working Group on Aflatoxins
TD_{50}	Carcinogenic Potency at 50%
WHO	World Health Organization

ABSTRACT

In Kenya, aflatoxicosis is a major public health concern and several outbreaks have occurred in the past due to the consumption of contaminated maize and maize products. Even when there are no reported cases of illness or deaths, it is believed that the consumer is constantly exposed to sub-lethal doses of the toxin above the established national maximum limit. The risk of developing liver cancer is six times higher in individuals exposed to aflatoxins. Epidemiological studies have also associated prolonged exposure of the mycotoxins with stunting and impaired growth in children due to protein malabsorption.

Much of the flours and flour mixes used for feeding especially children in Nairobi almost always contain maize and are usually purchased from small scale millers found widespread in the county. These millers do very little quality control if any.

This study was designed to assess the levels of aflatoxin contamination of flours from these small enterprises and its effect on growth when fed to rats. Questionnaires were administered to 107 Micro, Small and Medium Enterprises (MSMEs) to collect data on the nature, diversity and usage of milled flour products they supply. A total of 32 flour samples of maize, sorghum, finger millet and groundnut flours were collected from a selection of the interviewed MSMEs to test for levels of aflatoxin and to use to prepare contaminated rat pellets. Wistar rats were fed on the prepared pellets for a period of 21 days during which their weight, length and daily food consumption were recorded. Regression

analysis was done to determine correlations between variables. After the 21 days, a post mortem was done on the rats and their livers extracted for histopathological examination.

The aflatoxin levels in the 32 flour samples ranged from 2,190.30 ppb – < 1 ppb. Three (3), 6 and 5 out of 8 of maize flour, sorghum and groundnuts samples respectively collected had aflatoxin levels above the Kenya Bureau of Standards maximum limit of 10 ppb. Groundnut flour had the highest mean aflatoxin level contamination at 304.51 ppb. The mean aflatoxin level in maize flour, sorghum and millet flours was 59.73 ppb, 39.21 ppb and 34.80 ppb respectively. Regression analysis showed a significant negative correlation between weight gain of rats and consumption of aflatoxin contaminated feed. Amount of food consumed was also negatively correlated to ingestion of aflatoxin contaminated feed. Increase in length was not significantly correlated to consumption of aflatoxins. The histopathological examination of the rat livers showed fatty degeneration, cell outline alteration, nuclear changes, all signs of liver cell injury and necrosis.

The study established that flour products supplied by MSMEs in Nairobi County are contaminated with aflatoxins and are possible causes of poor growth, liver damage and necrosis.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Aflatoxins, after being identified in 1960, are to date the most intensively researched mycotoxins in the world; reason being that they have demonstrated potential carcinogenic and other toxicological effects in both humans and in laboratory animals (Massey et al., 1995). Aflatoxins are potent and toxic naturally occurring microbial carcinogens produced primarily by some strains of *Aspergillus flavus* and by most, if not all strains of *Aspergillus parasiticus*, as well as related species *A. nomius* and *A. Niger* (Groopman et al., 1988).

The natural habitats of *Aspergillus* genus include the soil, decaying vegetation and grains undergoing microbiological deterioration. Whenever conditions are favourable for growth, all types of organic substrates are susceptible to Aspergillus invasion (Baranyi et al., 2013). Aspergillus colonization can occur before harvest and during storage (Klich, 2007). Crops are particularly susceptible to infection following exposure to high humidity and high temperature in the environments (Cotty et al., 2007), damage from stressful conditions such as drought, insect or rodent infestation (a condition that lowers the barrier to entry), and poor genetic adaptability of plant variety to climate (Wu & Khlangwiseta, 2010).

Commodities most prone to aflatoxin contamination include cereal grains (maize, sorghum, millet, rice and wheat), oilseeds (groundnuts, soybean, sunflower and

cottonseeds), spices (chillies, peppers, coriander and turmeric), pulses and tree nuts such as almonds, walnuts, and coconut (Varga et al., 2009). Milk, eggs and meat products are found contaminated with aflatoxins if the animals have been fed on aflatoxin contaminated feeds (Prandini et al., 2009) .

In Kenya, aflatoxicosis is a major public health concern and several outbreaks have occurred in the past due to consumption of contaminated maize and maize products. In 2004, aflatoxin contaminated maize and maize products with levels of up to 800 ppb, resulted in the death of 125 people out of the reported 317 ill cases (Azziz-Baumgartner et al., 2005). The districts most affected were Makueni and Kitui in Eastern Province of the country. This was rated as one of the severest cases of acute aflatoxicosis observed in the world. Unseasonal early rains during harvest in February of the same year led to storage of maize under humid conditions conducive for *Aspergillus* growth (CDC, 2004). Besides the 2004 outbreak, other less severe outbreaks in Kenya occurred in 2005 and 2006, resulting in deaths of 30 and 9 persons respectively (Muthomi et al., 2009; Mwihia et al., 2008; Probst et al., 2011).

1.2 STATEMENT OF THE PROBLEM

The Micro, Small and Medium Enterprises (MSMEs) in Kenya have progressively penetrated the cereal and flour sector and have become popular with the public consumer. While large cereal and flour producing companies are located mainly in Industrial Area, MSMEs are mainly in residential estates (Onyango et al., 2014) with a higher concentration in low income estates.

A study on staple food consumption patterns among urban residents in Nairobi reported that consumption of maize meal from posho millers as compared to packaged, commercially milled maize flour sold in retail outlets was highest among the urban poor and in households headed by less professional individuals (Muyanga et al., 2006). However, this trend may be changing evidenced by the growing number of MSMEs dealing in cereal milling and flour supply as well as the successful penetration of their products into big retail supermarkets. One reason for this changed trend could be the fact that Nairobi residents are becoming more health conscious and are more aware of the nutritional benefits of less refined cereal products (Kang'ethe, 2011). These MSME cereal or flour dealers also supply a greater diversity of products such as maize, sorghum, millet, groundnuts, amaranth, cassava, dried green leafy vegetables and silver cyprinid (omena) flours, which when mixed into composite flours, are a preferred choice for many Kenyans.

A study by Aflacontrol project in 2010 analyzed maize samples from wholesalers, retailers and open air vendor from parts of western and eastern Kenya. Mean levels of contamination were higher in samples from the eastern sites, with a maximum of 1,633 ppb, 163 times higher than the allowed level of contamination for total aflatoxin level (Aflacontrol, 2010). In Kenya, exposure to aflatoxin has been reported to be primarily through ingestion of contaminated milled products as has been shown in the case of groundnut flour (Mutegi et al., 2013).

Though the Kenya Bureau of Standards has set a limit of maximum aflatoxin concentration in food crops at 10 ppb, aflatoxin contamination of food above this limit continues to be a health concern. Unlike in the large commercial millers where strict quality control measures are adhered to, MSME millers and flour traders only carry out simple procedures on their raw materials such as cleaning and dusting (Kang'ethe, 2011) but no chemical quality control tests like aflatoxin test are done on the raw material received. These enterprises often prepare composite flours from pure milled flours according to the wishes of the buyers. These composite flours are believed to be very nutritious and are used to prepare thin cereal based gruel/porridge for children and sick adults. However, controlled studies on aflatoxin levels in these porridge flours that are supplied by MSMEs in Nairobi County and their impact on growth has not been assessed. As stated by Muyanga et al. (2006), adult consumption of ugali from maize meal purchased from these posho millers is popular especially among the urban poor and would be a significant source of aflatoxin exposure to individuals.

With regard to children, the transition from breast milk to possibly highly contaminated weaning porridge is of significance. According to Wild (2007), "the nature of the weaning food, the relative quantities as compared with breast milk, and the duration of weaning before introduction of family foods have been reported to impact on the amount of aflatoxin exposure at this potentially critical period in life."

1.3 JUSTIFICATION.

Children are particularly affected by aflatoxin exposure which is associated with stunted growth and delayed development. Furthermore, due to the ability of aflatoxins to cross the placental barrier, exposure can cause genetic defects at foetal stages itself (Maxwell, et al, 1989). A study in Taiwan found Aflatoxin DNA adducts in 57.5% of placenta and cord blood samples (Hsieh & Hsieh, 1993). Kenya has high stunting rates of 35% and malnutrition rate (Global Acute Malnutrition [GAM]) stands at 7% (KNBS and ICF Macro, 2010). In order to be effective, interventions aimed at improving the nutritional status of children must therefore address the issue of aflatoxin contamination of foods.

Ascertaining that porridge flours are safe and do not negatively affect growth and nutrient metabolism due to liver dysfunction is critical. A systematic study of aflatoxin levels in products supplied by MSME millers in Nairobi County and its impact on health would be of great value in assessing the burden of disease in the county attributable to aflatoxin exposure.

1.4 OBJECTIVES

1.4.1 Main Objective

To assess the levels of aflatoxins in milled products from MSMEs and its effect on the growth of rats.

1.4.2 Specific Objectives

1. To determine the nature, diversity and usage of milled flour products from MSMEs in Nairobi County.

2. To determine factors contributing to aflatoxin contamination and levels of aflatoxins in milled products from MSMEs within Nairobi County.

3. To determine effect of feeding on aflatoxin contaminated flour on growth.

4. To determine hepatic histopathological manifestations due to feeding rats on aflatoxin contaminated flour.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

In many parts of the world, cereals make up the staple food of many communities and are relied upon as a major source of energy. They are eaten everyday and sometimes as often as in every meal and are frequently used to make porridges for both children and adults. Lack of diet diversification in some of these communities leads to overreliance on cereals whose production and marketing significantly affect the food security status of a region. Such cereals vary from country to country and typical examples include maize, wheat, millet, sorghum, barley and rice. These cereals are preferred because they are readily available, inexpensive and can remain intact when stored for extended periods.

However, it is these cereals that are most prone to infestation by mycotoxin producing fungus. Mycotoxins are varied and exhibit diversity in their mode of action. The major mycotoxins include aflatoxins, trichothecenes, ochratoxins, fumonisins and zearalenone. Of these, aflatoxins have been identified as highly toxic and carcinogenic compounds. The International Agency for Research on Cancer (IARC, 2002) has classified naturally occurring mixtures of aflatoxins as Class 1 human carcinogens. Contamination of cereals by aflatoxins is therefore a major health concern especially in communities that rely heavily on them as staple foods.

In Kenya, maize constitutes the major staple food in most households. Average daily consumption is at 400g per person (Muriuki & Siboe, 1995) and annual consumption per

capita has been estimated to be 98 kilograms (Snipes & Kamau, 2013). Aflatoxin contamination of maize leads to illness and even death, as has occurred severally in the country (Mwihia et al., 2008). In the most fatal outbreak of 2004, individual daily exposure was estimated at 50,000 µg/day of Aflatoxin B_1 (Probst et al., 2007). Diverse intervention strategies are therefore needed to control and prevent future hazardous contamination.

Although presence of aflatoxins in maize has received the greatest attention in Kenya due to it being a staple food, contamination is not limited to it. Groundnuts are also very prone to contamination (Mutegi et al., 2013) as well as other cereals such as millet and sorghum (Okoth & Ohingo, 2004), all of which are more than often found in the diets of Kenyans. Currently, the flour milling industry in Kenya is steadily growing and a more effective mycotoxin surveillance system is needed to ensure safety of flour products.

2.2 MICROBIOLOGY OF AFLATOXINS

Aflatoxins are toxic secondary metabolites produced by at least 20 species assigned to three sections of the *Aspergillus* genus. The three sections, as shown in Fig. 1 are *Flavi, Nidulantes and Ochraceorosei* (Baranyi et al., 2013; Varga et al., 2009).

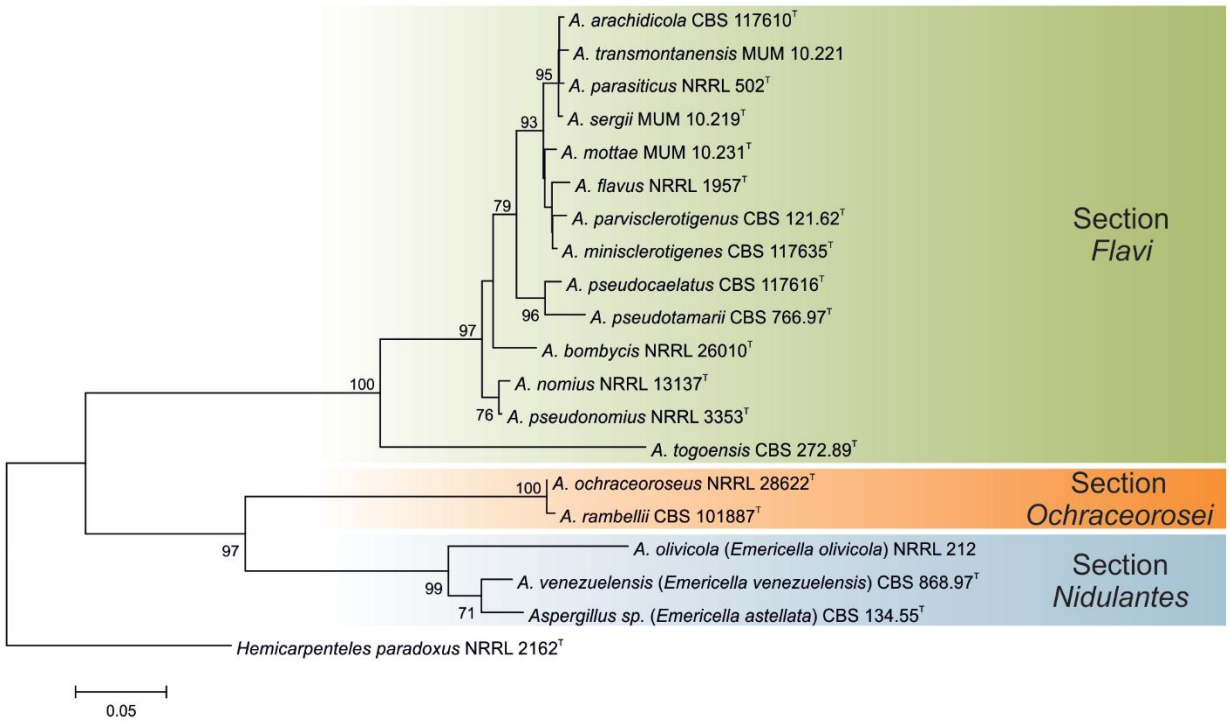


Figure 1: Phylogenetic Tree of Aflatoxin Producing Fungi
Source: Baranyi et al., 2013

There are four naturally produced aflatoxin strains denoted as B_1, B_2, G_1 and G_2. The B and G designation refers to the colour of fluorescence they produce under ultraviolet light (B for blue and G for green). The numbers (1 and 2) indicate their relative migration on a thin-layer chromatographic plate. Two additional groups referred to as M_1 and M_2 are the metabolic products of B_1 and are found in milk and other dairy products. The former two were discovered in the milk of lactating animals fed on aflatoxin contaminated feed; hence the M designation (Diener & Davis, 1969).

Figure 2 shows the chemical structure of these six major aflatoxins. Their structures are quite similar and their molecular formulas as established from elementary analyses and mass spectrometric determination (Cornell University, 2014,

http://www.ansci.cornell.edu/plants/toxicagents/aflatoxin/aflatoxin.html) are:- B1: C17 H12 O6, B2: C17 H14 O6, G1: C17 H12 O7, G2: C17 H14 O7

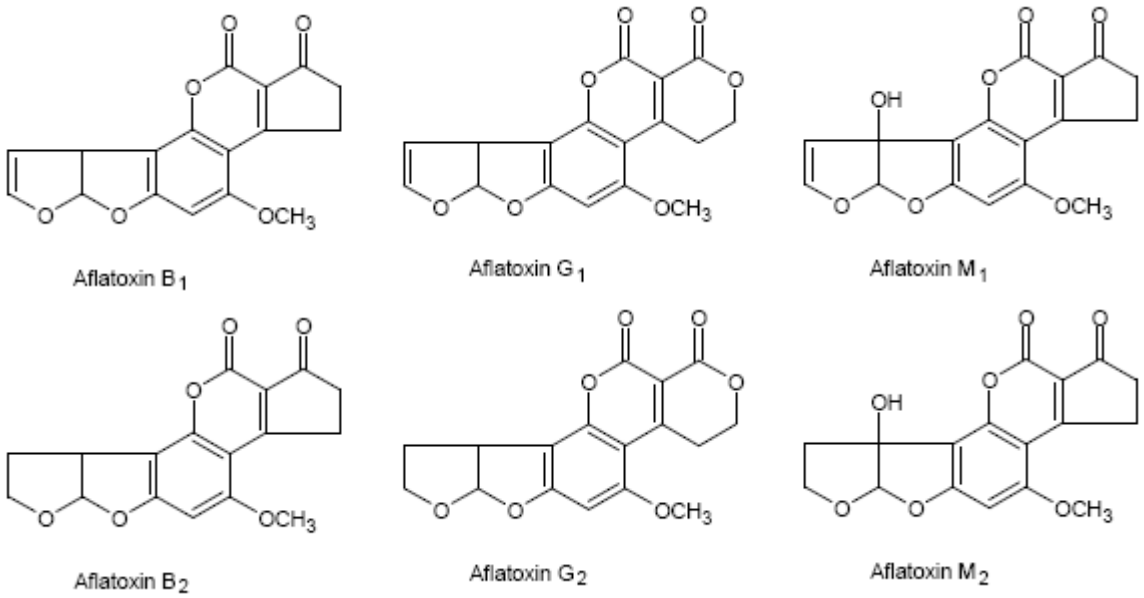


Figure 2: Chemical Structure of Aflatoxins B1, B2, G1, G2, M1 and M2
Source: http://www.mycotoxins.info/myco_info/science_moa.html

There are over a dozen other structural aflatoxin analogs (Baranyi et al., 2013). Aflatoxin B_3 is a metabolite of *A. flavus* while aflatoxin D_1 has been found in maize that was ammoniated. Aflatoxin P_1, Q_1, B_{2a} and G_{2a} are mammalian biotransformation products of the major four metabolites (Varga et al., 2009).

Of the above, aflatoxin B_1 (AfB_1) is the most toxic and potent naturally occurring carcinogen (IARC, 2002) and of all the aflatoxins, it is the highest produced by toxigenic strains (Baranyi et al., 2013).

2.3 CONTAMINATION AND PATHOLOGY OF AFLATOXINS

In 1985, the Food and Agricultural Organization (FAO) estimated that 25% of the world crops including many basic crops are contaminated with mycotoxins each year. Global losses of foodstuffs due to mycotoxins are in the range of 100 million tonnes per year of which 20 million tonnes comes from developing countries (FAO, 1996).

Fungal growth and aflatoxin production are the consequence of the interactions of the fungus, the host and the environment. Fungal infestation does not equate to aflatoxin production. Non toxin producing strains (atoxigenic strains) of *Aspergillus flavus* are known and have been used to control toxigenic strains (Cotty & Bhatnagar, 1994; Dorner, 2009).

Aflatoxin contamination is divided into two distinct phases: initial infection of the developing crop (often referred to as pre-harvest contamination) and subsequent contamination after maturation of the crop (often referred to as post harvest contamination) (Cotty, 2001). Both (Pre- and post-harvest contamination) are important in determining the levels of aflatoxin, while weather patterns influence the two contamination phases differently (Cotty & Jaime-Garcia, 2007).

In the first phase, infection by *Aspergillus spps* is highly favoured by drought stress, insect or rodent infestation or poor suitability of plant genotype to climate (Wu & Khlangwiseta, 2010). Environmental factors that favour infestation by aiding dispersal of fungal conidia include: high relative humidity, high soil and/or air temperature, high rates

of evapotranspiration, reduced water availability, nitrogen stress and crowding of plants (Klich, 2007).

In the second phase, contamination can occur any time between crop maturation and consumption (Cotty, 2001). Warm and moist conditions either in the field, during transportation or storage result in fungal infestation and possibly aflatoxin contamination of mature crops (Cotty, 1991). Even initially dry seeds when exposed to high humidity will be susceptible to fungal infestation due to increase in substrate moisture content (Cotty & Jaime-Garcia, 2007). Commodities with the highest risk of aflatoxin contamination are maize and peanuts (Freitas & Brigido, 1998).

Aflatoxin exposure occurs in humans mainly through ingestion of contaminated food. The exposure is described as acute, when high levels are ingested at once leading to illness with death occurring in some cases, or as chronic, when the individual is exposed to sub-lethal doses over a prolonged period of time. In tropical and sub-tropical countries such as Asia, Africa and South America, where environmental conditions favour growth of aflatoxin producing moulds, the threat of aflatoxicosis is quite high.

While ingestion of contaminated food products remains the main mode of exposure to humans, other modes do exist such as inhalation of the toxins (Brera et al., 2002; Dvorácková, 1976). Exposure to flour dust by workers in the milling industry therefore represents an occupational hazard which may lead not only to respiratory dysfuntion but also to diseases related to aflatoxin exposure (Awad, 2007; Meo, 2004).

The main metabolizing organ for aflatoxins is the liver, but this can also occur directly at the site of absorption, in the blood or in several extra-hepatic organs.

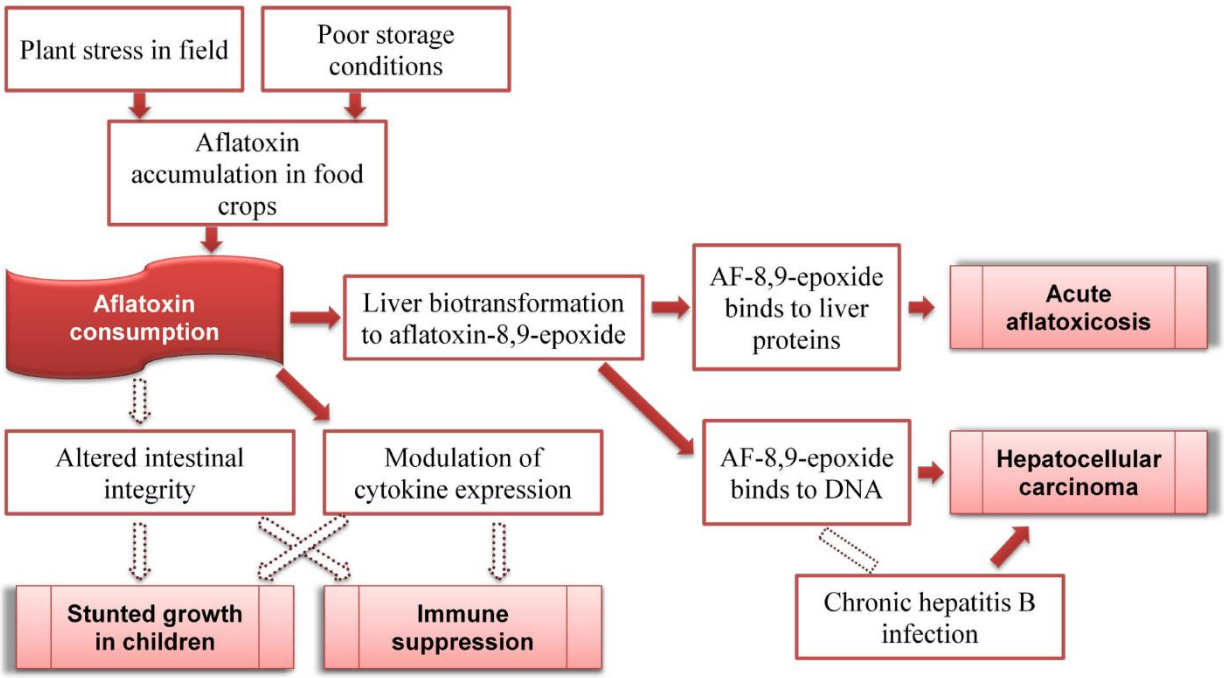


Figure 3: Aflatoxin Metabolism Pathways Resulting from Aflatoxin Consumption
Source: Wu, 2010 as cited in Wu et al., 2011.

Well established linkages in Figure 3 are denoted by shaded arrows while dotted arrows denote linkages that are relatively well established (Wu, 2010 as cited in Wu et al, 2011). Once ingested, aflatoxins may be transformed to their reactive form aflatoxin-8,9,-epoxide by the action of certain p450 enzymes. This reactive epoxide may either bind to liver proteins leading to liver failure (acute aflatoxicosis) or it may bind to DNA leading to aflatoxin induced hepatocellular carcinoma (Wu et al., 2011). Chronic infection with hepatitis B virus coupled with chronic aflatoxin exposure has been shown to lead to higher liver cancers through a synergistic interplay of the two (Liu & Wu, 2010).

Aflatoxin ingestion may follow two other pathways: alteration of intestinal function leading to stunted growth in children (Gong et al., 2008) and/or modulation of cytokine expression leading to immune suppression (Wu, 2010 as cited in Wu et al., 2011).

2.4 AFLATOXICOSIS: A GLOBAL OVERVIEW

According to FAO, countries that are situated between 40^{o}N and 40^{o}S are the most at risk of aflatoxin contamination. However, the greatest risk lies within the developing countries in tropical region which rely heavily on aflatoxin susceptible staple foods. Approximately 5 billion people in the developing world are exposed to aflatoxins (Williams et al., 2004).

In wealthy grain producing countries, economic resources are available for implementing safety regulations. Furthermore, the prices of maize and groundnuts are often dictated by aflatoxin contamination and this contributes to lower levels of exposure in these countries (Lizárraga-Paulin & Martinez, 2006).

Fatal aflatoxicosis has been reported in a number of countries. In 1974, 106 deaths resulting from aflatoxicosis were reported in Western India out of the 397 cases (Krishnamachari et al., 1975). In 2005, Nigeria reported 100 aflatoxicosis deaths (Wagacha & Muthomi, 2008). Outbreaks in Kenya have been several with the 2004 outbreak marking the highest number of deaths. According to a research carried out in 2010, aflatoxin contamination in Kenya was found to be more widespread than previously thought (Release & Foundation, 2011). This research identified widespread subsistence farming systems, lack of irrigation and inadequate drying and storage

facilities as factors that impede the prevention and detection of aflatoxin in crops in developing countries.

2.5 AFLATOXINS IN KENYA

Kenya has been the scene of recurrent outbreaks of aflatoxicosis with many outbreaks occurring due to consumption of contaminated home grown maize. As shown in Table 1, Eastern province has borne the brunt of it with most outbreaks occurring in Makueni and Kitui. As up to 2010, there had been a total 275 deaths in the country out of over 500 cases of aflatoxin poisoning.

Table 1: Cases of Aflatoxicosis Reported in Kenya from 1981 - 2010

Year	Number of cases	Number of deaths	Area of occurrence
1981	20	12	Machakos district
2001	-	12	Meru
2003	-	68	Thika, Kitui
2004	317	125	Makueni, Kitui
2005	75	32	Makueni, Meru, Kitui
2006	-	9	Makueni, Kitui
2007	84	21	Makueni
2008	6	2	Kitui (Mutomo)
2010	24	3	Makueni

Source: Mwang'ombe, 2014. Conference presentation at the 9th Biennial Scientific Conference and Exhibition, Faculty of Veterinary Medicine.

In 2010, Aflacontrol, a collaborative study with researchers from the Kenya Agricultural Research Institute (KARI) and the International Maize and Wheat Improvement Centre (CIYMMT), collected maize samples from the eastern and south western parts of Kenya to establish levels of aflatoxin contamination along the entire maize value chain.

The findings of the study revealed that aflatoxin contamination is very widespread in the country. Of the samples collected from farms in February 2010, 31% from the eastern sites and 40% from the south western region had aflatoxin levels above 10 ppb. The study established that maize stored by farmers after the harvest was more contaminated than that in the field. Furthermore, variation in aflatoxin levels from season to season and between regions was observed. The eastern region had samples with the highest contamination levels ranging from 0 to 1,400 ppb as compared to the south western region where contamination levels ranged from 0 – 700 ppb. The study established that little or no testing on maize is done in the markets to determine levels of aflatoxins.

Government measures to control aflatoxin contamination have leaned more on testing maize products at wholesale and retail outlets and withdrawing the contaminated batches (FAO/University of Nairobi, 2011). In 2010, the government mopped out contaminated maize from the eastern and coastal region of the country by purchasing the maize from farmers at a reduced price. While the strategy prevented the circulation and consumption of aflatoxin contaminated maize, it proved costly and methods to be used in disposing of mopped up maize have become an environmental concern (Lindahl, Delia, & Atherstone, 2014).

Currently, the recommended approach to the problem of aflatoxins is based on prevention rather than control or removal of already contaminated products (FAO/University of Nairobi, 2011). Furthermore, it is acknowledged that a multi-sectoral integrated approach

with involvement of all stakeholders along the agriculture and food chain from farmers to consumers is the most effective means of combating the problem.

In the recent past, great strides have been taken to combat aflatoxin contamination in Kenya. Research on preventive measures and control strategies have increased. This includes research on the use of bio-control strains of which the biocontrol product labelled 'Aflasafe KE01' is currently under testing in the country (Foodworld Media Team, 2014). Several regional bodies have also been established to combat the problem. Recently, in March 2014, the East Africa Community (EAC) established the Regional Working Experts Group on Aflatoxins (REWGA) whose mandate is to provide advisory and technical guidance to the EAC and to key stakeholders on the prevention and control of aflatoxins. The Partnership for Aflatoxin Control in Africa (PACA) was launched on October 31, 2012 by the African Union Commission (AUC) to coordinate and provide leadership to aflatoxin control efforts in Africa. Several aflatoxin control projects in Kenya are coordinated by this African Union body (PACA, 2014).

Despite these advances in combating aflatoxins in the country, much remains to be done. There is a "continued need for multidisciplinary and comprehensive research to inform policy and to test possible solutions" (Unnevehr & Grace, 2013).

2.6 TOLERABLE LEVELS OF AFLATOXINS

In 2008, the Codex Alimentarius Commission set a maximum level of 10 ppb for total aflatoxins for 'ready to eat' nuts (hazelnuts, almonds and pistachios). In an effort to align itself to the Codex Alimentarius, the European Union has in the recent past revised its

2006 regulation (USDA Foreign Agricultural Service, 2010). The current *Commission Regulation (EU) No. 1652/010* sets higher maximum limits for both AfB_1 and total aflatoxins than the previous 2006 regulation. It also covers a wider range of food products as compared to the Codex Alimentarius and categorizes foods into 'ready to eat' and 'for further processing'. Following this new Commission Regulation, maximum aflatoxin limits for ready to eat nuts is at 8ppb (AfB1) and 10ppb (total aflatoxins). Ready to eat corn has limits of 2 ppb (AfB1) and 4 ppb (total aflatoxin). In the United States, maximum limit of total aflatoxins in human food is set at 20 ppb while that of milk is 0.5 ppb (FDA, 2000). Kenya has adopted conservative tolerable aflatoxin levels in human food at 10 ppb and 5 ppb for total aflatoxins and AfB_1 respectively (KEBS, 2013).

2.7 HEALTH EFFECTS OF AFLATOXIN INGESTION

Aflatoxicosis is primarily a hepatic disease. When ingested, aflatoxin binds to liver proteins and can lead to several health related conditions such as acute and chronic aflatoxicosis, aflatoxin related immune suppression, liver cancer and liver cirrhosis (USAID, 2012).

Chronic low level exposure to aflatoxins, especially AfB_1, is associated with an increased risk of developing hepatocellular carcinoma (HCC) or liver cancer, as well as impaired immune function and malnutrition and stunted growth in children**.** According to the World Health Organization (WHO, 2008), HCC is the third leading cause of cancer deaths globally. HCC as a result of chronic aflatoxin exposure presents most often in persons with chronic hepatitis B virus and/or chronic hepatitis C virus infections (IARC, 2002). In fact, studies have shown that persons with hepatitis B infection who live with

chronic aflatoxin exposure have a risk of contracting liver cancer that is 30 times greater than people who are hepatitis B-negative (Kirk et al., 2006; Liu & Wu, 2010).

Globally it is estimated that aflatoxicosis contributes to between 4.6% and 28.2% of liver cancer cases (WHO, 2008). Each year, 550,000–600,000 new liver cancer cases are recorded worldwide, and of these, approximately 25,200–155,000 are attributable to aflatoxin exposure (Liu & Wu, 2010). The global liver cancer burden is primarily borne by Sub-Saharan Africa, Southeast Asia, and Western Pacific nations (USAID, 2012). The IARC GLOBOCAN 2008 (as cited in Wu et al., 2011) estimated liver cancer incidence in Kenya at 8.5 and 4.9 per 100,000 for males and females respectively. This was higher than the incidence of HCC for males in North America and Europe during the same year which was 6.8 and 6.5 per 100,000 respectively.

Studies have also shown that chronic exposure to aflatoxins may directly impair immune function and worsen human immunodeficiency virus (HIV) infection and other infectious diseases (Williams et al., 2004).

2.8 AFLATOXICOSIS AND LINKS WITH STUNTING AND KWASHIORKOR

Wagacha & Muthomi, (2008) reported that majority of inhabitants of sub-Saharan Africa are inclined to consume mycotoxin contaminated products by their low socio-economic status. Among population, children are among the most vulnerable group. They have smaller body weights and aflatoxin doses that might not affect adults may induce illness in them. Children also have more immature neurologic and immune systems that are more susceptible to toxin effects (Magnussen & Parsi, 2013).

A study by Gong et al. (2004) in Benin and Togo revealed that high exposure to aflatoxins critically affects growth and development of children. In these countries, aflatoxins contaminate staple foods particularly maize and groundnuts leading to high exposure throughout childhood (Gong, 2002). A cross sectional study in Kisumu by Okoth & Ohingo, (2004) investigating association between aflatoxin exposure and nutritional status of children found out that 31% of the children were malnourished. The number of children who were wasted and were being fed on flour contaminated with mycotoxins was highly significant.

Aflatoxins have also been linked to kwashiorkor, a disease caused by protein energy malnutrition. Kwashiorkor has some characteristics associated with the pathological effects caused by aflatoxin exposure in animals, but the link between aflatoxin exposure and kwashiorkor is not clear (Shephard, 2008). Despite these preliminary findings and the fact that aflatoxins have been found in the liver of children suffering from kwashiorkor, a strong cause-effect relationship between the two has not been established (Katerere, Shephard & Faber, 2008).

2.9 MICRO, SMALL AND MEDIUM ENTERPRISES IN KENYA

MSMEs are generally thought to play a crucial role in driving economic growth in both developing and developed countries (Beck, Demirguc-Kunt & Maksimovic, 2004). As a group, MSMEs generate more new jobs than large firms. They introduce innovative ideas, products, and business methods. In Kenya, MSMEs provide 80% of employment and contribute 40% to GDP (Mwarari, 2013) and therefore have their place in the new economic development strategy in Kenya vision 2030.

In view of this, the MSE Act passed in 2012 represents the first attempt in the country to consolidate the regulatory and institutional policy framework surrounding these enterprises (Ong'olo & Awino, 2013). The Act was a product of the collaboration between the Ministry of Labour and the SMEs stakeholders in Kenya with the objective of regulating, developing and revitalizing the sector. Among other criteria, categorization or definition of MSEs in the Act is based on number of employees and annual turnover of an enterprise (Table 2).

Table 2: Definition of MSEs according to the MSE Act, 2012

Business Entity	No. of Employees	Annual Turnover
Micro Enterprise	Less than 10 people	Not exceeding KES 500,000
Small Enterprise	More than 10 but less than 50	Between KES 500,000 to 5 million

Source: GoK

The 2012 Act does not provide criteria for defining a medium enterprise. Based on number of employees, a medium enterprise is considered to have 50-99 employees (Africa Centre for Open Governance, 2012).

The milling industry in Kenya has been characterized by rapid growth and many MSME traders have emerged throughout the country and have penetrated the industry. They offer lower prices, match up to customer preferences in a more direct and personal way, and offer a greater diversity of flour products (in pure form or as mixes). These MSME millers therefore have great potential for further growth. For this to happen, it is

important that the quality and safety of their milled products be continuously checked and upgraded especially with reference to aflatoxin contamination.

2.10 METHODS OF TESTING FOR AFLATOXINS

Detection of aflatoxin levels in food stuff such as cereal flours mainly employs analytical and immunological methods. Analytical methods include Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GS) while the immunological method is mainly the Enzyme-Linked Immunosorbent Assay (ELISA) (Gilbert & Vargas, 2003).

In many countries, especially in developing nations, ELISA has become the most common detection test for mycotoxins. Besides being portable for use in the field, ELISA is also rapid, simple, specific and sensitive (Zheng et al., 2005). The assay works by detecting and quantifying the presence of aflatoxin (the antigen) in a sample by using an enzyme labelled toxin and antibodies that are specific to the aflatoxin (ICRISAT, n.d.). The antibodies are first coated on the wells of the ELISA plate. The test sample and the enzyme labelled aflatoxin are then added to the wells. If the test sample has no toxins, the enzyme labelled toxin will bind itself to the antibodies coated to the wells. However, if aflatoxins are present, they will compete with the labelled toxins in binding to the antibodies. The wells are then washed and any unbound labelled enzyme is washed away. A substrate is then added and the intensity of the resulting colour is proportional to the amount of enzyme labelled aflatoxin-antibody complex in the well. This means that the greater the concentration of aflatoxins in the test sample, the lesser the colour intensity (ICRISAT, n.d.).

A research study by Zheng et al. (2005) validated the accuracy of ELIZA kits comparable to the use of HPLC in measuring the level of total aflatoxins ranging from 4 to 40 ppb.

2.11 USE OF RAT STUDIES FOR HUMAN AFLATOXICOSIS

Much of the published information on the effect of aflatoxins in man has been obtained from the study of rats (Kensler et al., 2011). Particular emphasis has been placed on acute toxicity of the B_1 fraction of the aflatoxin complex which are recognized as one of the most potent hepatocarcinogens (Butler & Neal, 1977; Kensler et al., 2011). Studies have also been done on a variety of other experimental animals with rabbits, ducklings, rainbow trouts, dogs and guinea pigs showing highest sensitivity to aflatoxin exposure (Wogan, 1966).

Wogan explains that the toxic effect of aflatoxins on animals depends on the duration of exposure, the dose and the test system. When administered acutely in high large doses, effects are lethal leading to formation of cancerous tumours in the liver and other tissues and ultimately death. When administered in smaller doses, histopathological changes in the liver and other organs are observed which may lead to development of tumours if exposure is chronic. These histopathological changes include necrosis with biliary proliferation. Other changes include fat accumulation, presence of parenchyma cells with enlarged nuclei and oval cell proliferation (Newberne & Butler, 1969).

In rats, the youngest animals are most susceptible to aflatoxin toxicity while mature females are considerably more resistant, a characteristic which seems to be lost during the latter stages of pregnancy (Newberne & Butler, 1969).

According to a previous research by Wogan (1965), the AfB_1 LD_{50} (as a single dose, oral route) of 21 days old male and female rats, is 5,500 µg/kg and 7, 400 µg/kg respectively. Death occurred within a period of 7 days and histopathological examination revealed gross liver damage.

Table 3 summarizes findings of a research by Wogan (1966) in which rats were fed on partially purified aflatoxins by stomach tube. The treatment lasted for 30 days after which the animals were fed on an aflatoxin-free diet.

Table 3: Rat Tumour or Lesion Incidence after Administration of Purified Aflatoxins

Aflatoxin level (ppb)	Feeding time (days)	Tumour or lesion incidence after 10 months of feeding on aflatoxin-free diet
150	30	100% incidence of cancerous tumours
75	30	80% incidence of cancerous tumours
37.5	30	100% incidence of precancerous lesions
15	30	80% incidence of precancerous lesions

The rats feed on the lowest aflatoxin level of 15 ppb developed lesions which could have progressed into cancerous tumours if the observation period had been prolonged past 10 months. Furthermore, the fact that tumours or lesions developed months after feeding on non contaminated aflatoxin feed shows that hepatoma can be induced by a brief initial low aflatoxin exposure and chronic exposure over a long period is therefore not required.

The Carcinogenic Potency Database (CPDB) by Gold et al. (as cited by Wogan, 1992) gives the Carcinogenic Potency (TD_{50}) of a diversity of species. The TD_{50} values, expressed as µg/kg body weight/day for Fischer rats are 1.3 (males) and 7.5 (females); for Wistar rats 5.8 (males) and 6.9 (females).

Gold et al. (1987) defines TD_{50} as follows:

For a given target site(s), if there are no tumors in control animals, TD_{50} is that chronic dose rate in mg/kg body weight/day which would induce tumors in half the test animals at the end of a standard lifespan for the species. Since the tumor(s) of interest often does occur in control animals, TD_{50} is more precisely defined as that chronic dose rate which will halve the probability of remaining tumor-free throughout the standard lifespan of the species (p. 237).

In the above CPDB, the standard lifespan of rats was set at 2 years (104 weeks). The conventional lifespan of rats and mice is usually between 90 – 110 weeks.

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY DESIGN

The study was cross-sectional in design with an analytical component. A survey of MSME millers and flour suppliers in Nairobi County was carried out to establish the nature and diversity of flours supplied in the market. The analytical component consisted of two parts: laboratory analysis of aflatoxins in flour samples and assessing the effect of aflatoxin contaminated flours on growth and liver tissue of laboratory rats.

3.2 STUDY SETTING

The survey was carried out in Nairobi County, the capital city of Kenya. The aflatoxin and in vivo analysis were carried out in the Department of Public Health, Pharmacology and Toxicology (PHPT), the Department of Food Science, Nutrition and Technology (FSNT) and the Department of Veterinary Pathology, Microbiology and Parasitology, all at the University of Nairobi, Upper Kabete Campus.

3.3 METHODOLOGY

3.3.1 The survey

A questionnaire was prepared (Ref. Appendix I), pretested in Thika and administered to MSME flour suppliers located within Nairobi County. Four research assistants were trained and involved in data collection. Based on the division of Nairobi County into seventeen constituencies by the Independent Electoral and Boundaries Commission (Infotrack East Africa, n.d.), each enumerator covered at least three constituencies over a

period of four days and interviewed the MSME millers and flour suppliers located in those areas.

The study focused on flour products supplied to and consumed by the public. The inclusion criteria for the survey therefore, were MSMEs whose core business is to supply milled flour products. Based on this, questionnaires were administered to businesses that:- mill and sell flour products to consumers; take raw grains to millers for milling and then sell the milled products to customers; and those that buy already milled flour products which they sell to consumers.

Excluded from the survey were MSME millers who solely mill on contract or on order and therefore do not keep stock of the grains or flours and do not supply flours to consumers.

The aim of the survey was to collect data on the nature and diversity of milled products supplied by MSMEs, their usage, the average daily turnover of the flour products and whether quality control tests are carried out on the cereal grains before milling.

3.3.2 Sample size calculation

According to the Nairobi City Council records, there were 168 registered MSME posho mills in Nairobi County in 2013. There were no records on registered MSME retailers who sell flour products. The population of MSMEs in Nairobi County that supply flour products to the public was therefore considered to be 168. A sample size of 117 was

calculated at confidence level of 95% and margin of error of 5%. The sample size estimation for descriptive statistics was used (Hulley et al., 2001) where:

$n = (z_\alpha / E)^2 P (1-P) = 384$

New n (correction for finite population) $= \dfrac{n}{1 + \frac{n-1}{N}} = 117$

Where	n	=	sample size
	N	=	Population size (168)
	z_α	=	Critical value of Z statistic at 95% confidence level (1.96)
	P	=	P is the expected proportion who have the characteristic of interest (0.5)
	E	=	margin of error at 5%

The characteristic of interest was MSMEs who supply flour products to the public and therefore fit within the inclusion criteria. In order to arrive at the largest sample size possible, P = 0.5 was used.

Due to logistical constraints, a total of 107 MSMEs were interviewed. Being a count data, this number of MSMEs (107) was sufficient to carry out correctly statistical analysis. This is because count data follows normal distribution and therefore the exact calculated sample size need not be achieved. For normally distributed variables, a sample size of 30 is considered to be adequate.

3.3.3 MSMEs sampling method

Convenient sampling was done from MSMEs found along established pathways or roads in Nairobi County and to these, questionnaires were administered. Questionnaires were not administered to MSMEs located deep within estates or slums.

3.3.4 Flour sample collection and testing for aflatoxins

3.3.4.1 Flour sample collection

Samples of flour products that are prone to aflatoxin contamination and are frequently used to make composite flour porridge for children and adults were collected. Summary of the survey data revealed four flour products that meet these criteria: maize flour, sorghum, finger millet and groundnut.

Random sampling was used to select the MSMEs from whom flour samples would be collected as shown in Figure 4. A total of 32 flour samples (8 from each type of flour product) were collected and tested for aflatoxins in replicate.

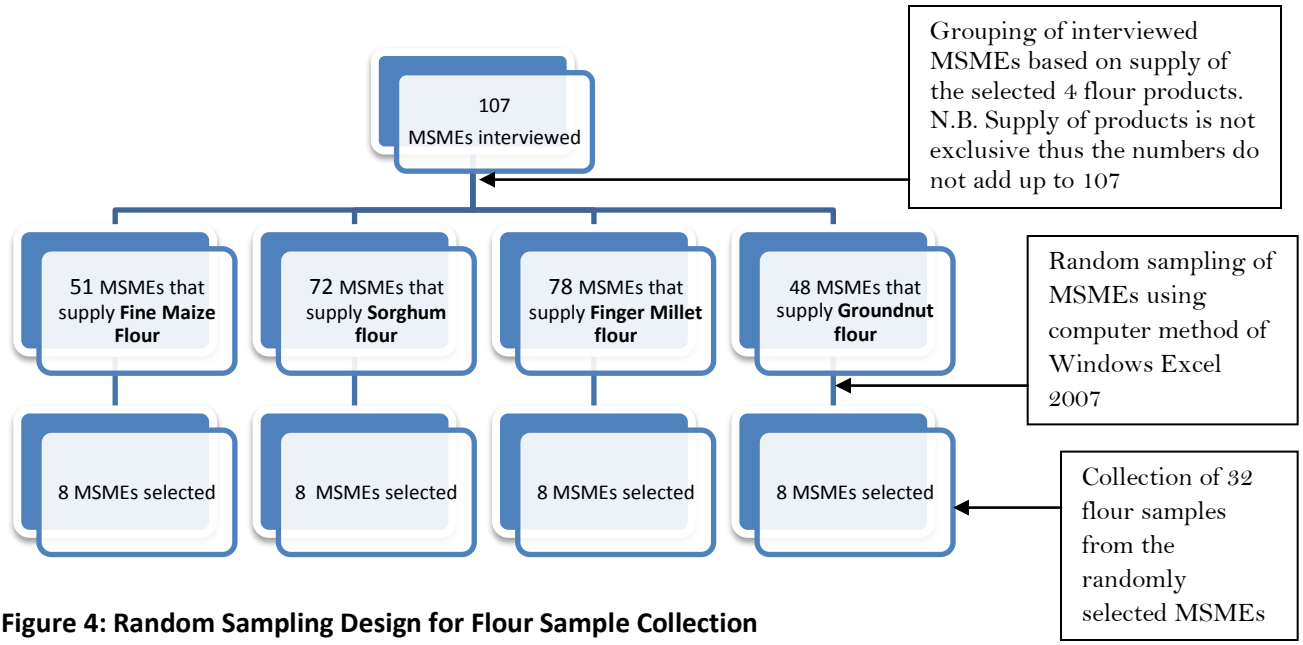


Figure 4: Random Sampling Design for Flour Sample Collection

To ensure collection of representative samples, sampling using a spike was done from the top, mid and bottom of all bags/containers. The flour samples were packed in brown kraft paper bags, sealed with masking tape and sample codes given on the outside of the bag.

The flour samples were stored in a cool and dry area to preserve and maintain the flours in the same state as when collected.

3.3.4.2. Determination of levels of aflatoxin contamination

The samples were then tested for total aflatoxins using the rapid screening Aflatoxin ELISA Test Kit (Helica Biosystems, Cat. No. 941AFL01M – 96). All reagents were brought to room temperature before use. Extraction of the samples was done using 70% methanol with a ratio of sample to extraction solvent of 1:5. Dilution wells and an equal number of antibody coated microtiter wells for each standard and sample to be tested were placed on a microwell holder. Six standards of the following concentrations were used: 0.0 ppb, 1.0 ppb, 2.5 ppb, 5.0 ppb, 10.0 ppb and 20.0 ppb.

200µl of the conjugate was dispensed into each dilution well. Using a new pipette tip for each, 100µl of each standard and sample were added to appropriate dilution wells containing conjugate. Mixing was done by priming the pipettor at least 3 times. The location of each standard and sample was recorded and ascertained throughout the testing.

Using a new pipette tip for each, 100µl of contents from each dilution well was transferred to a corresponding antibody coated microtiter well. This was followed by incubation of the microtiter wells at room temperature for 15 minutes. The contents were then decanted into a discard basin. The microwells were washed by filling each with distilled water and then decanting the water into a discard basin. A total of 5 washes were

done. Residual water from these micowells was removed by tapping them face down on a layer of absorbent paper.

100µl of substrate reagent was then added to each microwell followed by incubation at room temperature for 5 minutes. The wells were covered to avoid direct light. 100µl of the stop solution was then added to each microwell in the same sequence and at the same pace as the substrate was added. Finally, with a microtiter plate reader using a 450nm filter, the Optical Density (OD) of each microwell was read and recorded.

3.3.5 Feeding of rats with aflatoxin contaminated composite flour

3.3.5.1 Preparation of composite flour rat feed

Three different categories of composite flours were prepared each having different levels of aflatoxins:

1. Low level aflatoxin composite flour mix (0 – 10 ppb)
2. Medium level aflatoxin composite flour mix (11-50 ppb)
3. High level aflatoxin composite flour mix (> 50 ppb)

The aflatoxin levels and cut-offs were set following the Wogan (1966) research model on rats. The low level was set at a maximum of 10 ppb to agree with the KEBS aflatoxin limits.

As shown in figure 5, each category of the above composite flours consisted of a mixture of fine maize flour, sorghum, finger millet, groundnuts and silver cyprinid flour. The first four products were added in a ratio of 5:5:5:2 respectively to make a mixture weighing approximately 2.6 kg in each category, an amount sufficient to feed six rats for 21 days.

This ratio was based on trends observed in the survey and practiced by the MSMEs millers when making composite flours.

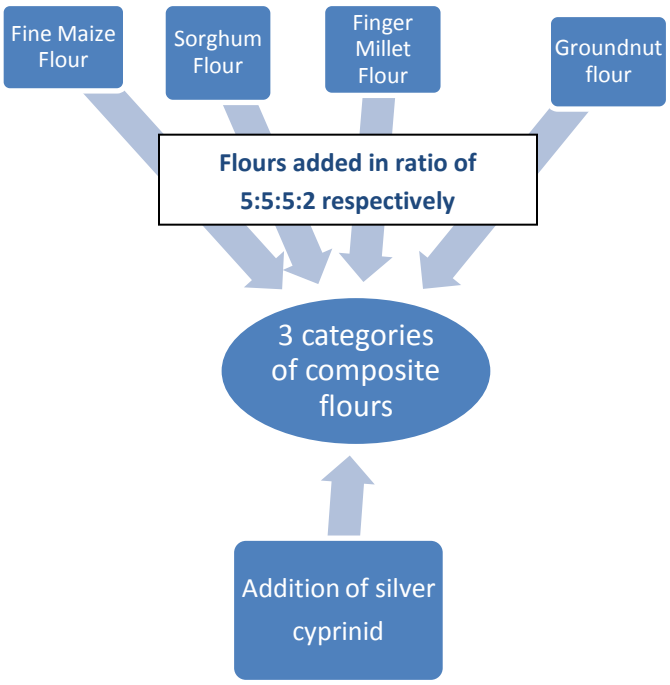


Figure 5: Schema Showing Preparation Method of Composite Flours

The low level mix was prepared by mixing the low level aflatoxin contaminated flours following the above ratio. The medium level mix was likewise prepared by mixing intermediate level aflatoxin contaminated flours and the high level mix with highly contaminated flours.

Silver cyprinid flour, which is also frequently added to porridge flour mixes, was then added to increase the protein content of the flour mixes to 17% to match the protein requirements of rats (Meireles et al., 1999). This was achieved by first determining the crude protein as total nitrogen by the Kjeldahl method (AOAC, 1999) in each of the

prepared three categories of composite flours and in silver cyprinid flour that had been purchased. The Pearson square method was then used to arrive at the amount of silver cyprinid flour to add in order to arrive at 17% protein content in each of the categories of composite flours. However, the protein content obtained was approximately 14%. This is because two different silver cyprinid flour purchases were used to increase the protein content. The Pearson Square calculation used silver cyprinid protein content of 66.82% which corresponded to the first purchase only. The second purchase, whose protein content was not established, must have had a lower protein content.

3.3.5.2 Determination of proximate composition and aflatoxin content of composite flours

The composite flours now made up of five flour products: fine maize, sorghum, finger millet, groundnuts and silver cyprinid were thoroughly mixed by hand to obtain a homogenous mixture. Proximate composition of the three categories of composite flours was then carried out according to AOAC methods (AOAC, 1999).

Aflatoxin content of the three composite flour mixes was also determined using Total Aflatoxin Detection ELISA kits Helica Biosystem as before. The aflatoxin testing was done on the raw and cooked composite flours.

3.3.5.3 Rat feeding

Eighteen laboratory rats of the species *Rattus norvegicus*, strain Wistar were used in establishing the effect of aflatoxin contaminated flour on rat growth. The rats, all born within the same week and with an average age of 2 weeks at the beginning of the study

were selected from the PHPT animal room. The rat feeding study design was as shown in Figure 6.

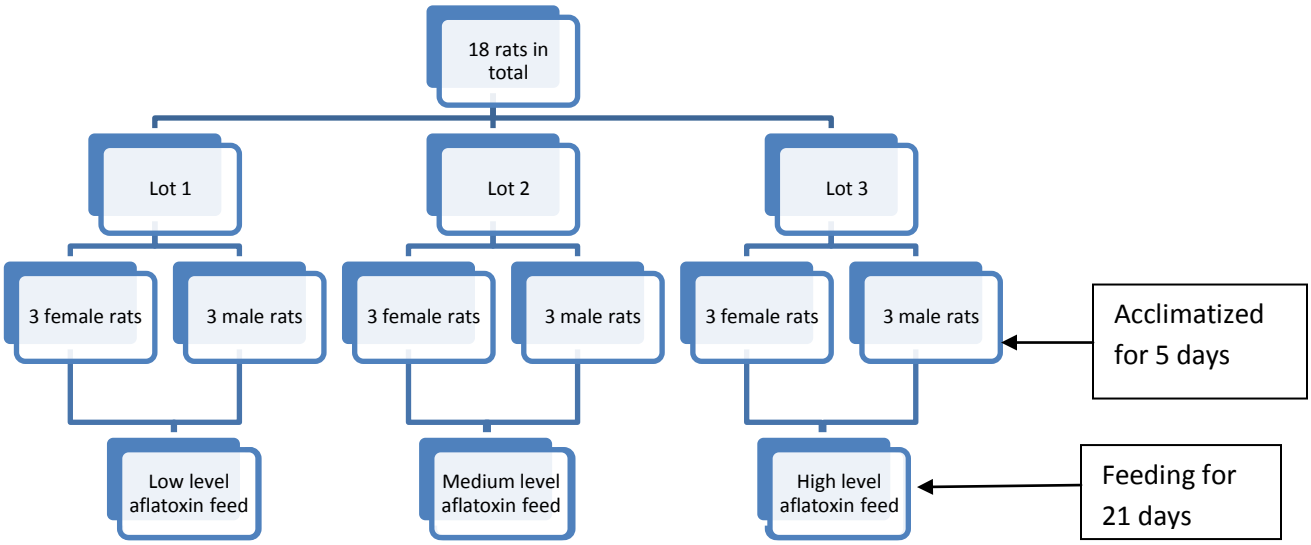


Figure 6: Design of Rat Feeding Study

The rats were housed in plastic cages of dimensions 14"×9"×8" (three rats in each cage) inside a well-ventilated room at 22±2ºC with a 12-hr L:D cycle and humidity ranging from 67-71%. The cages were lined with wood shavings. The above conditions are in accordance with internationally recognized guidelines (National Research Council, 2011; National Health & Medical Research Council, 2013) for housing rats for research.

The weight and length of the rats were measured at the beginning of the feeding regime and at 2 days interval for a period of 21 days.

The rats were fed on cooked composite flours to simulate cooking of porridge for human consumption. Pellets were moulded from the cooked flours to facilitate feeding and measurement of leftover food. The cooking was done every two days and was standardized by adding 254g portion of each of the three categories of composite flours to 200ml - 300ml of boiling water to make a thick porridge or 'ugali'. Round flat pellets were made from the thick porridge or 'ugali' and dried in a Memmert 1977 oven at 40 ^{o}C for approximately 20 hours. The weight of feed given daily to the rats and left-over the following morning were determined using a Mettler PM4600 Delta Range weighing balance. The amount of pellet food consumed by the 3 rats in each cage was then calculated. Average amount of food consumed by each rat in each cage was calculated by dividing this amount by three. It was assumed that all the rats in a cage consumed equal amount of feed. The control lot was fed on the low aflatoxin composite flour.

3.3.6 Histopathological examination of the rat livers

After 21 days, the rats were anaesthetized using 99.9 % diethyl ether and bled to collect blood samples for future liver function tests. This procedure was carried out by a trained animal laboratory technician. Post mortem of each of the rats was immediately carried out by trained pathologists at the Department of Veterinary Pathology, Microbiology and Parasitology. A histopathological examination of the rat livers was then carried out. The rats were dissected, examined systematically and all morbid changes encountered recorded appropriately. The liver was specially targeted in this exercise. Upon evisceration, the liver was freed, wiped dry and weighed using an electronic balance and weight recorded.

Tissues samples from the liver were collected from all the rats and put into 10% formalin. After fixation, the samples were dehydrated using graded alcohol and embedded in paraffin wax. Sections of 4 - 5 µm thickness were cut, stained with hematoxylin and eosin and examined under a light microscope. All microscopic lesions were recorded appropriately.

The rat carcasses were disposed of by incineration at the Department of Pathology, Faculty of Agriculture and Veterinary Sciences, University of Nairobi.

3.4 BIOSAFETY PROCEDURES TO MITIGATE EXPOSURE TO AFLATOXINS

The principal investigator and university personnel assisting in the study adhered to accepted biosafety measures. Precautions were taken to avoid any ingestion and inhalation of aflatoxin or skin contact with aflatoxin when handling the aflatoxin contaminated flour samples in the research laboratories. These involved the use of gloves and covered laboratory coats, use of a mouth-nose mask when necessary, hand washing after handling of contaminated flour samples or after exiting the laboratories, no eating or drinking in the laboratories, use of mechanical pipetting devices when testing for aflatoxins in flours samples and proper signage of flour products and rate cages (University of Chigago, 2012).

3.5 ETHICAL CONSIDERATIONS

Consent from respondents was sought before administration of the questionnaire and names of millers and flour suppliers were kept confidential. Approval for the use of

laboratory rats was granted by the Biosafety, Animal Use and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi.

3.6 DATA MANAGEMENT AND ANALYSIS

The survey data was coded, inputted and cleaned for analysis using Statistical Package for Social Sciences (SPSS 16.0). Descriptive analysis of survey results was done to provide general information about the variables, followed by inferential analysis.

Data from the rat feeding study was analysed using Generalized Estimation Equations (GEE) using Genstat version 8. GEE was used because the rat data represented repeated measures which are non independent due to clustering in time. Standard regression analysis assumes independence and would therefore have resulted in erroneous interpretations.

GEE regression analysis generated estimates of parameters taking weight gain, length gain and food consumption as the response variables against the medium and high levels of aflatoxin contamination. The control or reference factor was the response of the female rats to the low aflatoxin composite flour feed. The estimates of parameters were generated using rat measurements of weight, length and food consumption recorded in 7 visits over the 21 days study period.

CHAPTER FOUR

RESULTS

4.1 NATURE, DIVERSITY AND USAGE OF MILLED PRODUCTS BY MICRO, SMALL AND MEDIUM ENTERPRISE MILLERS IN NAIROBI COUNTY

One hundred and seven (107) MSME flour millers and suppliers were interviewed from 15 constituencies of Nairobi County. Table 4 shows the number of survey respondents interviewed from each constituency in the county.

Table 4: Number of Survey Respondents Interviewed from Each Constituency in Nairobi County

Constituencies of Nairobi County	No. of MSMEs interviewed
Westlands	7
Dagoretti North	2
Dagoretti South	7
Langata	8
Roysambu	4
Kasarani	6
Embakasi South	1
Embakasi North	3
Embakasi Central	15
Embakasi East	9
Embakasi West	10
Makadara	10
Kamukunji	11
Starehe	1
Mathare	13
Total	**107**

Of these 107 respondents, 93.6% were microenterprises having less than ten employees, 5.5% were small enterprises having ten to forty nine employees while only 0.9% were medium enterprises having fifty to ninety nine employees. Fifty four percent (54%) of all the MSMEs sell less than a bag of flour every day while only 6% sell ten and above bags of flour per day (Figure 7).

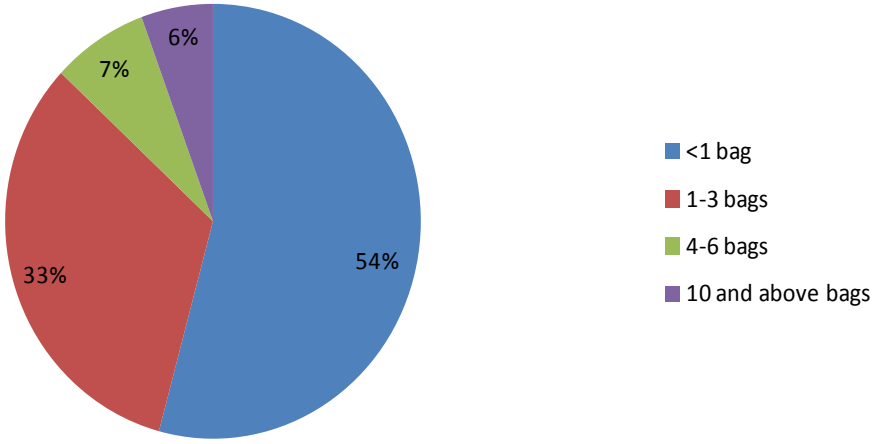


Figure 7: Percentage Average Daily Sales in Bags (90kgs) by the MSMEs

The core businesses and proportion of market share of all the interviewed MSMEs is shown in Figure 8. Majority (56%) of the MSMEs mill and sell flour products through either wholesale or retail outlets, 23% of MSMEs buy and sell already milled flour products while 18% of the MSMEs take raw grains to millers to mill for them the grains and then sell the milled flour products through either wholesale or retail outlets. Less than 2% of MSMEs do a combination of business activities, reflecting specialization of the MSMEs in their core business. Of the 56% that mill and sell flour products, only 12% farm their cereals for milling. The majority (88%) buy their cereal grains from farm

producers or middlemen located within Nairobi County or in other parts of the country. Nyamakima, Dagoretti and Kangemi are some of the areas within Nairobi County where MSMEs often purchase their cereals.

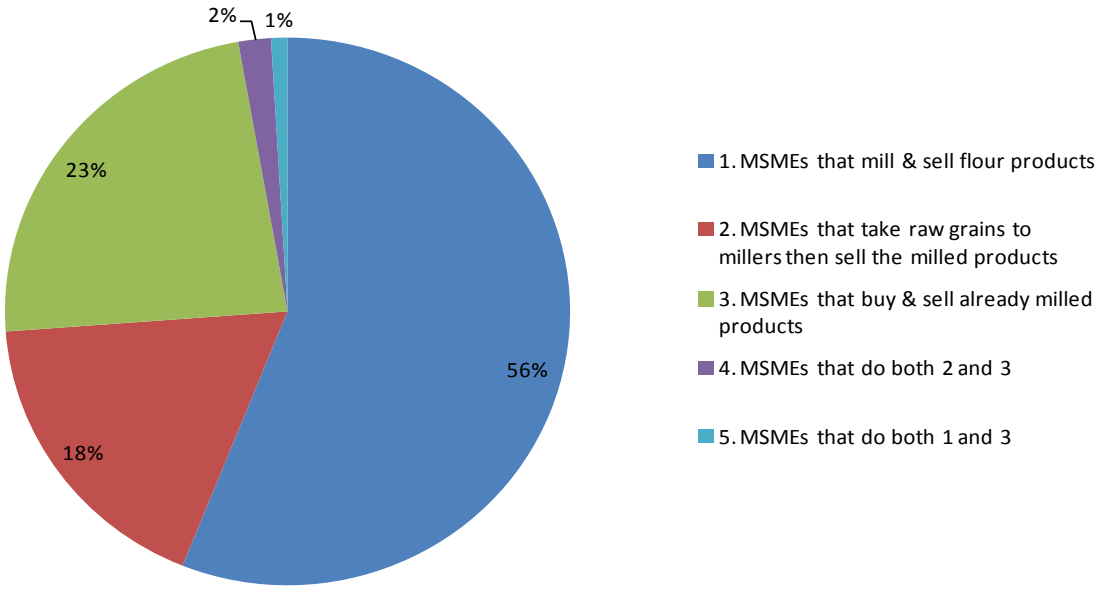


Figure 8: Types of Core Businesses and Share of Market of MSME Flour Millers and Suppliers within Nairobi County

The variety of flour products, more than twenty different products, ranging from cereal to legume flours are sold by these MSMEs. Flour products identified in the market were: - maize meal, maize flour/heho/unga baridi, sorghum, finger millet, pearl millet, foxtail millet/mkombi, cassava, wheat, groundnuts, silver cyprinid/omena, stinging nettle/thabai, amaranth/terere, soya beans, other beans (black beans/njahi, kidney beans, wairimu beans, rosecoco beans, saitoti beans, nyayo beans), green grams/ndengu, pigeon

peas/mbaazi, cow peas and cow pea leaves/kunde, pumpkin seeds, flax seeds, oats, rye, rice and sim sim.

As shown in Table 5, Micro enterprises supply over 90% of the wide range of flour products in the market while Medium enterprises supply only one type of flour product.

Table 5: Percentage of Type of MSME supplying Flour Products

Flour Product	Percentage (%) of Type of Enterprise Supplying Flour Products		
	Micro Enterprise (1-9 employees)	Small Enterprise (10-49 employees)	Medium Enterprise (50-99 employees)
Maize meal	95	5	0
Maize flour	90	8	2
Sorghum flour	94	6	0
Millet flour	93	7	0
Cassava flour	93	7	0
Wheat flour	86	14	0
Protein rich flours (G/nuts, soya, omena)	95	5	0
Stinging nettle flour	93	7	0
Amaranth seed flour	95	5	0
Green gram and pigeon/cow peas flour	90	10	0
Beans flour	100	0	0

Figure 9 shows the most popular pure flour products sold by MSMEs. Being the staple food of Kenyans, the most commonly supplied product is maize meal (88%) followed by finger millet flour (71%) and sorghum flour (66%). Pearl millet, cassava and protein dense flours such as groundnut, soya bean and silver cyprinid flours are also commonly supplied.

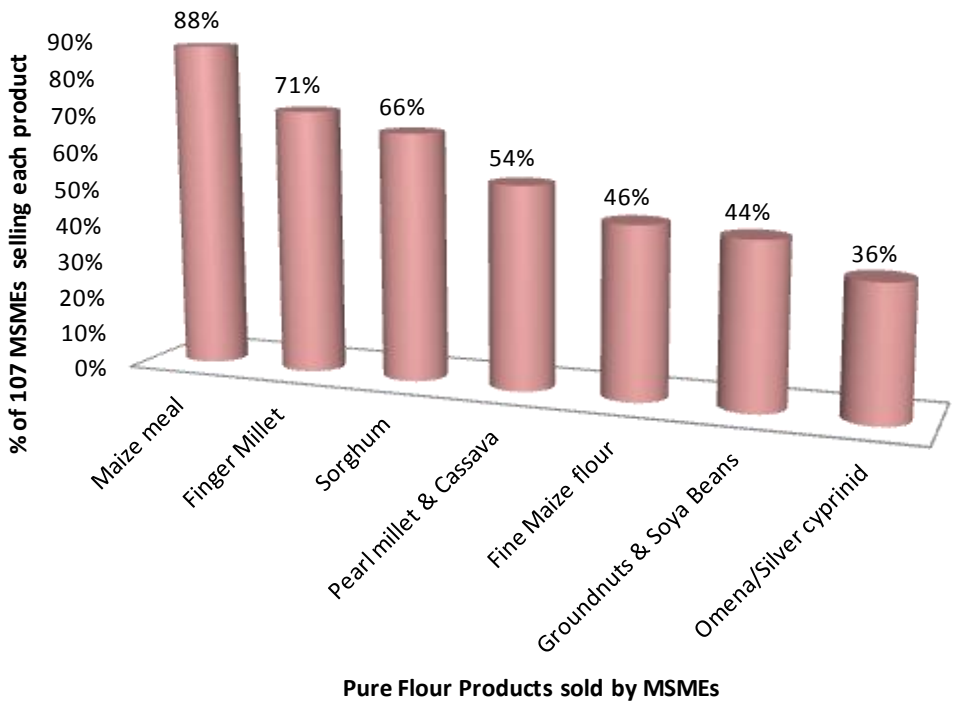


Figure 9: Shares of the Common Pure Flour Products Sold by the Interviewed MSMEs in Nairobi County

Sixty two percent (62%) of the MSMEs sell composite flours while 38% do not. The composites are quite varied with regard to the mix ingredients but sorghum, finger millet and maize flours are commonly used. Below is a listing of some flour mixes in terms of ingredients.

1. Maize flour+sorghum+finger millet
2. Maize flour+sorghum+finger millet+groundnuts
3. Maize flour+sorghum+finger millet+groundnuts+silver cyprinid
4. Maize flour+sorghum+groundnuts+amaranth
5. Sorghum+finger millet+amaranth
6. Sorghum+finger millet+groundnuts/silver cyprinid
7. Sorghum+finger millet+cassava+soya

8. Maize meal+soya+amaranth
9. Maize flour+sorghum+pearl millet+finger millet+groundnuts/soya
10. Maize meal+maize flour+cassava
11. Maize flour+sorghum+finger millet+cassava
12. Maize flour+finger millet+rice
13. Maize flour+sorghum+groundnuts+amaranth+rice
14. Pearl millet+finger millet+groundnuts+silver cyprinid+stinging nettle+wheat
15. Groundnuts/silver cyprinid+soya+cassava+wheat

There is no standard formula across the industry for the types of flour mixes and proportion of ingredients added to prepare the mixes. However, four flour ingredients are common in mixes used to make porridge for both children and adults. These are maize flour, sorghum, finger millet and groundnuts. The proportion used in mixing these products varies but the cereals tend to be mixed in almost equal ratio while less is added of the protein rich groundnut flour. Thus, to make a 1 kg flour mix, the flour ingredients may be added in the following quantities: 300 g of maize flour, 300 g of finger millet flour, 300 g of sorghum flour and 100 g of groundnut flour. Silver cyprinid flour is also frequently bought and added to mixes to make a high protein content porridge for children. The quantity added of each of the three cereal flours may then be 250 g, 150 g of groundnut flour and 100 g of silver cyprinid flour.

Some MSMEs however sell some standard mixes which they refer to as "Nutritious Mix", "Uji Mix", "Baby Mix" etc. Sour flours prepared by the addition of a souring agent are also available in the market.

MSMEs who supply high protein content flour such as groundnut, silver cyprinid and soya flours, demonstrated knowledge that such flours should be consumed by children who are above 1.5 or 2 years due to possible allergic reactions.

With regard to customer consultation on appropriate flour type to purchase based on needs, 54% of respondents said that customers consult them while 46% said they do not. Customers who consult refer to various usages and needs for buying the flours either as mixes or pure products. These include making porridge for normal (healthy) children and adults, for children with rickets, for malnourished children, for diabetic adults or for adults with arthritis. For each of these conditions, certain flour ingredients are commonly used to prepare flour mixes that are believed to meet the nutritional needs of the ailing person.

Concerning quality control procedures on cereal grains employed by the interviewed enterprises, 64% of the measures involve simple removal of impurities through sieving, sorting or winnowing. 30% of the measures are sensorial - observation, touching or even biting of cereals mainly to check level of dryness, maturity or presence of pesticides or undesirable spots. The remaining 6% include measures such as adding rodenticide tablets to keep off pests/rodents and demanding high quality grains from suppliers.

4.2. LEVELS OF AFLATOXIN CONTAMINATION AND FACTORS CONTRIBUTING TO AFLATOXIN CONTAMINATION

The aflatoxin levels in thirty two samples of four different flour products are as shown in Table 6. Three (3), 6 and 5 out of 8 of maize flour, sorghum and groundnuts samples

respectively collected had aflatoxin level above the Kenya Bureau of Standards maximum limit of 10 ppb. Groundnut flour had the highest mean aflatoxin level contamination at 304.51 ppb. The mean aflatoxin level in fine maize flour, sorghum and millet flours was 59.73 ppb, 39.21 ppb and 34.80 ppb, respectively. However, Kruskal Wallis statistical test showed that there was no significant difference in aflatoxin contamination levels between the means of the four flour products (P>0.05).

Table 6: Levels of Aflatoxin (ppb) in Pure Flour Samples Collected from MSMEs

Flour Samples collected from different MSMEs	Levels of Aflatoxin (ppb) in Flour Product Samples			
	Maize Flour	Sorghum	Finger Millet	Groundnuts
MSME 1	341.91	80.48	248.79	2,190.30
MSME 2	97.47	72.85	7.75	135.93*
MSME 3	30.68*	65.92	7.69	48.35
MSME 4	3.48	39.74	6.31	34.88
MSME 5	3.10	26.67	3.07	26.63
MSME 6	1.21	22.78	2.08	<1
MSME 7	<1	5.26	1.66	<1
MSME 8	<1	<1	1.03	<1

The levels with an asterix were detected in flour samples from Small enterprises. All the other aflatoxin levels were detected in samples from Micro enterprises.

Of the samples with levels above the tolerance of 10 ppb, 87% were from Micro enterprises while 13% were from Small enterprises.

The survey data on factors that would influence aflatoxin levels in flour products from the MSME millers from whom the 32 pure flour samples were collected was further analysed. Cross tabs were done to determine storage and quality control factors that

significantly influence the levels of aflatoxins in the pure flour samples. The aflatoxin levels were categorized as Low (0-10 ppb), Medium (11-50 ppb) and High (>50ppb) which corresponds to the aflatoxin level categorization used in preparing the rat feed. The Fisher's Exact value of diverse factors cross tabbed against the above aflatoxin levels are tabulated in Table 7.

Table 7: P Value of Various Quality Control and Storage Variable Cross Tabbed against Low, Medium and High aflatoxin Levels of Collected Pure Flour Samples

Independent Variable: Aflatoxin levels	Quality Control & Storage variables	Values in each variable	Fisher's Exact Value (P>0.05)
Low (0-10 ppb) Medium (11-50 ppb) High (>50 ppb)	Type of core business		0.833
	Type of Quality Control (QC) tests done on raw material	• Dirt Removal-Sorting, sieving, winnowing • Sensorial QC-feeling, smelling, tasting • Use of moisture meter	0.670
	Cleaning of raw material	• Yes • No	0.301
	Drying of raw material	• Yes • No	0.107
	Packaging material of raw material	• Jute bag • Plastic material- bag, container • Paper material	0.432
	Awareness of raw material storage conditions prior to purchase	• Yes • No	0.274
	Storage conditions monitored where raw materials are stored	• Room kept dry and/or cool/ventilated • Cleanliness • Use of wooden planks • Control of insects • 2 or more of the above conditions • None	0.083
	Length of storage of raw material	• Days – 1 week • 2-3 weeks • 1 month • 2-3 months	0.788
	Cleaning of storage room	• Yes • No	0.437
	Packaging material of milled flour product	• Jute bag • Plastic material- bag, container • Paper material	0.449
	Storage conditions monitored where milled flour product is stored	• Room kept dry and/or cool/ventilated • Cleanliness • Use of wooden planks • Control of insects • 2 or more of the above conditions • None	0.504
	Length of storage of milled flour product	• Days – 1 week • 2-3 weeks • 1 month • 2-3 months	0.715

From the P values above, none of the quality control and storage condition variables significantly influenced the variation in levels of aflatoxin contamination in the collected pure flours.

4.3. PROXIMATE ANALYSIS OF RAT FEEDS

The proximate composition of the three categories of rat feed compared to commercial pellets and the recommended standard diet of rats is shown in Table 8. There was no significant difference in the proximate composition of the three categories of composite flours prepared for the study (P>0.05).

Table 8: Proximate Composition of Different Rat Feeds

	Proximate Composition of Rat Feeds				
	Prepared for the study				
	Low aflatoxin composite feed	**Medium aflatoxin composite feed**	**High aflatoxin composite feed**	**Rat pellets from Unga Ltd**	**Basic recommended standard diet for rats***
Moisture (%)	8.90	8.92	8.98	10.34	
Fat (%)	5.17	7.46	5.45	3.73	Not <4.50
Protein (%)	14.31	13.98	13.55	2.98	17.0
Ash (%)	2.97	3.25	3.31	7.22	Not >8.0
Fibre (%)	2.12	3.09	2.17	7.79	Not >6.0
Carbohydrates (%)	66.53	63.30	67.12	67.94	58.00
Energy (K/cal)	369.89	376.26	371.85	317.25	

*The protein content is based on the study by Meireles et al. (1999). The fat, ash, fibre and carbohydrate figures represent the content in rodent standard diet for biomedical research (Lab Diet, 2013 http://www.labdiet.com/cs/groups/lolweb/@labdiet/documents/web_content/mdrf/mdi4/~edisp/ducm04_028021.pdf)

4.4. LEVELS OF AFLATOXINS IN THE THREE CATEGORIES OF COMPOSITE FLOUR

The levels of aflatoxins in the three categories of cooked and uncooked composite flour rat feeds are shown in Table 9.

Table 9: Aflatoxin Levels In The Uncooked and Cooked Flours of the Three Categories of Rat Feeds

	Aflatoxin levels (ppb)		
	Low aflatoxin composite flour	**Medium aflatoxin composite flour**	**High aflatoxin composite flour**
Uncooked flour	7.05	40.49	148.45
Cooked flour	6.90	45.51	148.45

The difference in the level of aflatoxins between the uncooked and cooked composite flours was minimal. The cooking method therefore did not significantly alter the contamination level of the aflatoxin.

4.5 REGRESSION ANALYSIS OF RAT DATA

Measurements of weight, length and food consumption recorded in 7 visits of the 21 days study period (Appendix III) were analysed using GEE statistical test. The regression analysis generated estimates of parameters with weight gain, length gain and food consumption as response variables.

Table 10 shows estimate of statistical parameters taking weight gain as the response variable. Weight gain was significantly and negatively associated with both levels of aflatoxins. However, the medium aflatoxin level showed a greater negative association with weight gain than the high level of aflatoxin. Weight gain was not significantly

associated with the sex of the rats; being female or male did not significantly influence weight gain.

Table 10: Estimate of Parameters Taking Weight Gain as Response Variable

Parameter	Estimate	s.e.	t (114)
Constant	113.2	14.3	7.92
Sex Male	5.06	3.07	1.65
Afla ppb 2 (Medium Level)	-19.51	4.71	-4.14
Afla ppb 3 (High Level)	-13.91	3.84	-3.62
At Confidence Interval = 0.95 or α = 0.05			

Table 11 shows the estimate of statistical parameters taking length gain as response variable. From the t values, neither sex nor the two levels of aflatoxin showed a significant association with length gain; though negative, the associations were not significant.

Table 11: Estimate of Parameters Taking Length Gain as Response Variable

Parameter	Estimate	s.e.	t (114)
Constant	21.96	1.83	11.98
Sex Male	-0.250	0.480	-0.52
Afla ppb 2 (Medium Level)	-0.072	0.782	-0.09
Afla ppb 3 (High Level)	-0.932	0.621	-1.50
At Confidence Interval = 0.95 or α = 0.05			

The estimate of statistical parameters taking food consumption as response variable is shown in Table 12. Both levels of aflatoxin contamination are significantly and negatively associated with food consumption. However, the Medium level is more

negatively associated with food consumption than the High aflatoxin level. The sex is significantly and positively associated with food consumption. Therefore, as compared to the females, the males consumed more food during the 21 days of study.

Table 12: Estimate of Parameters Taking Food Consumption as Response Variable

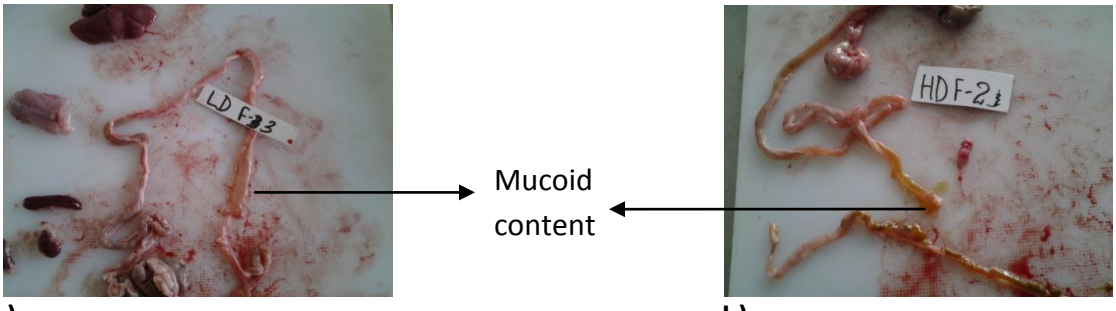
Parameter	Estimate	s.e.	t (114)
Constant	8.24	2.18	3.77
Sex Male	1.253	0.387	3.24
Afla ppb 2 (Medium Level)	-4.461	0.508	-8.79
Afla ppb 3 (High Level)	-2.144	0.488	-4.40

At Confidence Interval = 0.95 or α = 0.05

The full and detailed GEE regression analysis output is attached as an appendix (Ref. Appendix IV)

4.6. RAT HISTOPATHOLOGIC MANIFESTATIONS

At post mortem, the intestines of 16 out of the 18 rats had mucoid content (Figure 10).



a) **b)**

Figure 10: Extracted Intestine Sections at Post Mortem: (a) From Female Rat Fed on Low Aflatoxin Feed; (b) from Female Rat Fed on High Aflatoxin Feed. Both Show Mucoid Content

The histopathological examination revealed that in the rats fed on low aflatoxin feed, the effects of the aflatoxin were observed in selected foci where hepatocytes were abnormal. In these foci, fatty changes and necrosis were evident.

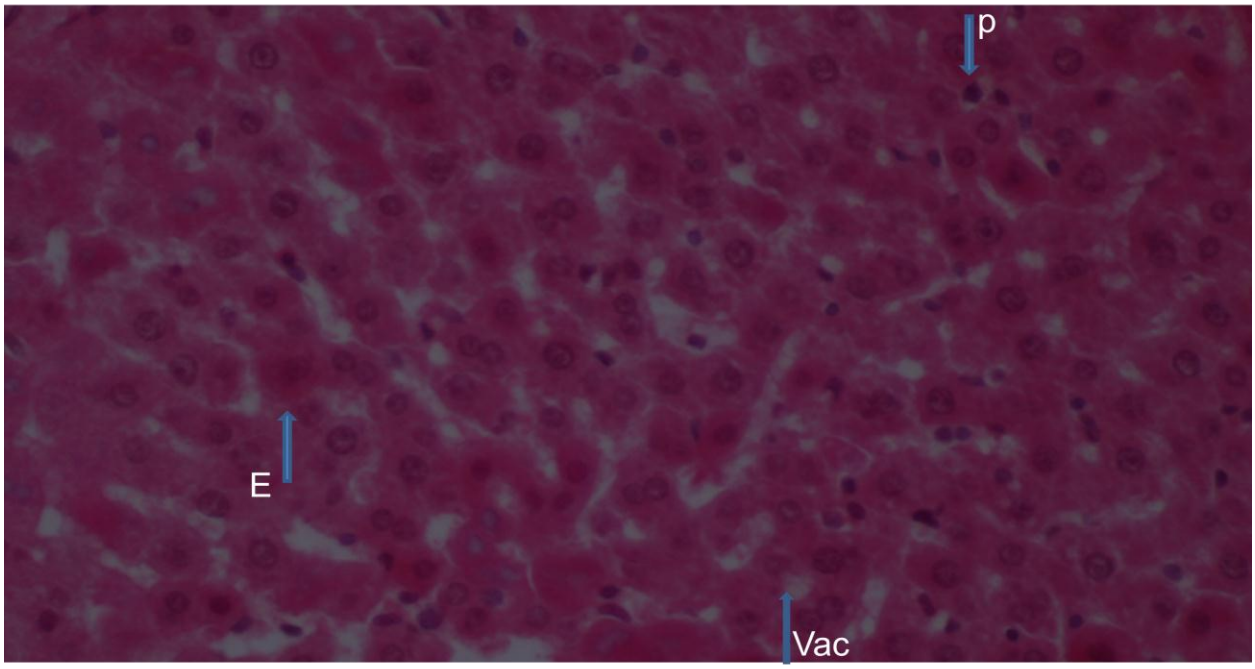


Figure 11: Liver Section from Rat Fed on Low Aflatoxin Feed Showing Unstainable Vacuoles (Vac) a Sign of Fatty Degeneration and Eosinophilia (E) and Pyknosis (P) Characteristic of Necrosis

In the rats fed on medium aflatoxin feed, grey (pale) patches the size of pin-point were observed; with most hepatic cells being abnormal. Cells with intact nuclei, but with many cytoplasmic vacuoles possibly indicating fatty changes were observed, depicting a reversible change following injury. The other cells showed reduced nuclei size. Staining blue or black indicated pyknosis which is a sign of cell death or necrosis.

In the rats feed on high aflatoxin feed, the entire liver had signs of injury. Cytoplasmic and fatty changes and eosinophilia were observed, all indicative of cell injury. Majority of the cell had nuclear/necrotic changes representing pyknosis, karyolysis, loss of cell outline or even absence of cells.

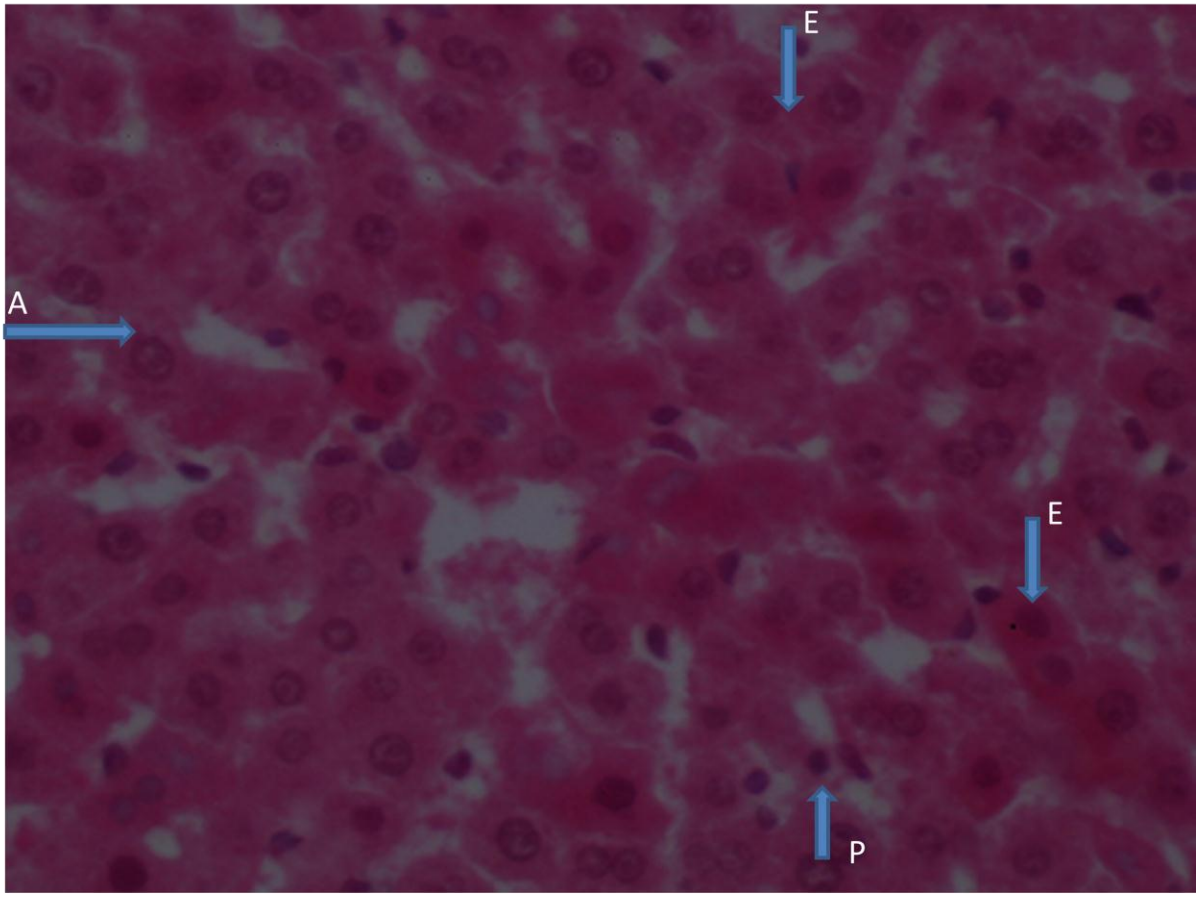


Figure 12: Liver Section From Rat Fed on High Aflatoxin Feed Showing Necrosis Characterized by Nuclear Changes; Pyknosis (P), Loss Of Cells/Karyolysis/Acellular (A) and Cytoplasmic Changes mainly Eosinophilia (E) X 400

No signs of inflammation were seen in all the 18 Wistar rats feed on aflatoxin feed.

Though the livers of rats fed on high aflatoxin feed had the highest liver:body weight

ratio (Table 13), a possible sign of greatest liver damage and necrosis, there was no significant difference in the mean liver:body weight ratios of the rats feed on low, medium and high aflatoxin composite flours. Therefore, using the liver:body weight ratio as an indicator of liver damage, there was no significant difference in liver damage in the 3 categories of rats.

Table 13: Liver:Body Weight Ratios of Rats at Post Mortem

Aflatoxin Level	Rat	Liver Weight at Post Mortem	Body Weight at Post Mortem	Liver:Body Weight Ratio	Mean
Low Dosage	Female 1	6.72	161.11	0.0417	**0.03995**
	Female 2	4.43	131.19	0.0338	
	Female 3	5.33	123.43	0.0432	
	Male 1	6.47	159.93	0.0405	
	Male 2	7.99	184.13	0.0434	
	Male 3	5.77	155.70	0.0371	
Medium Dosage	Female 1	5.80	125.67	0.0462	**0.04548**
	Female 2	5.23	116.86	0.0448	
	Female 3	6.11	138.29	0.0442	
	Male 1	4.78	103.45	0.0461	
	Male 2	4.48	109.40	0.0410	
	Male 3	5.40	106.72	0.0506	
High Dosage	Female 1	5.94	120.66	0.0492	**0.0488**
	Female 2	5.83	120.07	0.0486	
	Female 3	5.97	120.62	0.0495	
	Male 1	6.40	140.52	0.0455	
	Male 2	9.07	182.70	0.0496	
	Male 3	8.25	163.82	0.0504	

CHAPTER FIVE

DISCUSSION

5.1 NATURE, DIVERSITY AND USAGE OF MILLED PRODUCTS FROM MICRO, SMALL AND MEDIUM ENTERPRISES IN NAIROBI COUNTY

From the survey data, most of the MSMEs interviewed were micro enterprises at 93.6%. This agrees with a World Bank report (2012) which cites a survey of MSMEs in Kenya done in 1999 which reported that above 90% of all general enterprises were microenterprises. Though these micro enterprises have less than 10 employees and have an average daily turnover of less than one 90 kg bag, they have successfully infiltrated the flour market, especially within the densely populated lower income areas of Nairobi County such as Eastlands. These findings agree with Muyanga et al. (2006) who reported that the consumption of cereal products from posho millers in Nairobi is highest among the urban poor. Nevertheless, Kang'ethe (2011) observed that there is an increased demand for these products in Kenya owing to the growing consumer awareness of their health benefits. Thus, these posho millers are also found in higher income areas of the county such as Langata and some parts of Westlands such as Parklands.

Of the interviewed MSMEs, most (56%) mill their own cereal grains and then sell to the public. These millers mostly buy the cereal grains from either maize producers or middlemen. The second highest category of MSMEs (23%) that deal in flour products are those that buy already milled flour products and subsequently sell to consumers. Only 18% of MSMEs take their own cereal grains to be milled and then sell the flour products. The above findings shows that there are quite a number of flour suppliers in the market

who have no knowledge and/or control over the quality and storage of raw cereal materials utilized in making the flour products they sell. They simply retail flour products and their role in quality control would be to maintain quality or food safety of products as bought from the millers. It was observed that these retailers are located around millers and the type of products they sell is limited to what the millers supply.

The diversity of flour products sold by MSMEs is wide with over twenty different products ranging from cereals to legumes. This included maize meal, finger millet flour, sorghum flour, pearl millet flour, cassava flour and protein dense flours made from groundnuts, soya beans and silver cyprinid. However, this diversity is highly attributable to the Micro enterprises. Over 90% of the enterprises supplying the various flour products are Micro enterprises while only 10% or less are Small enterprises. Medium enterprises mostly specialize in supply of only one product, maize flour.

This diversity allows for mixing of different types of flours, with over 60% of the interviewed enterprises selling composite flours. A study by Kang'ethe (2011) reiterates this fact by stating that these enterprises undertake value addition through blending of a variety of different flours resulting in flour mixes that are more nutritious. The nutritional value of these flours was confirmed in our study by the analysis done on the silver cyprinid flour and the cooked composite rat feeds. The protein content of the silver cyprinid flour that was purchased was 66.82% which when added to other flours can significantly increase the overall protein content of the flour mix.

These composite flours are bought for consumption as porridge by both children and adults with the aim of maintaining or improving health. The survey revealed that there are no standardized formulae in making these flour mixes; the type and ratio of products mixed is left to the discretion of either buyer or seller or both upon consultation. Some suppliers sell flour mixes comprising of up to eight different products and therefore questions of nutrient interactions arise.

5.2 AFLATOXIN LEVELS IN FLOUR SAMPLES

Analysis of the aflatoxin levels detected in the 32 samples did conform with existing literature. Freitas & Brigido (1998) state that commodities with the highest risk of aflatoxin contamination are maize and peanuts (groundnuts). Likewise in this study, groundnut and maize flours were found to have the highest aflatoxin levels of 2,190 ppb and 342. ppb respectively, which are far above the Kenya Bureau of Standards maximum limit of 10ppb. Most of the contaminated samples with aflatoxin levels above 10 ppb were from Micro enterprises. This can be explained by the fact that over 90% of MSMEs in Kenya are Micro enterprises and therefore most of the flour samples were collected from them. A Kruskal Wallis statistical test however showed that there was no significant difference in the means of the four different types of flour samples collected. Therefore, in order to simulate a porridge feed that consumers often prepare, all the four different flour products were mixed in this study.

5.3 FACTORS CONTRIBUTING TO AFLATOXIN CONTAMINATION OF FLOURS

The Fisher's Exact value ($P > 0.05$) of diverse factors that could have an influence on the degree of aflatoxin contamination showed that none of the factors was significant. The reason for this could be that most of these MSMEs buy already contaminated cereal or flour products and therefore any quality control measure they employ would not make a significant difference in the level of aflatoxin contamination. These MSMEs do not have control over prior harvesting and handling practices that can prevent initial contamination of products and therefore no action at their level can significantly affect aflatoxin levels.

5.4 EFFECT OF AFLATOXIN CONTAMINATED FLOURS ON GROWTH OF RATS AND LIVER PATHOLOGY

GEE statistical analysis showed a significant negative correlation between weight gain as well as food consumption by Wistar laboratory rats with aflatoxin contamination. However, in both variables (weight gain and food consumption) the rats fed on medium aflatoxin contaminated flour mix of 45.51 ppb showed a greater negative correlation compared to the rats fed on the high aflatoxin contaminated flour mix of 148.45 ppb. This could be because the rats fed on medium aflatoxin contaminated flour mix ate poorly for the first 2-3 days after which feeding amounts (in grams) increased (Ref. Appendix III, C). The poor feeding at the beginning of the study could have been caused by the particle size of the pellets. Initially, these pellets were crushed into fine particles with the intention of facilitating feeding. However, this was stopped when it was observed that the

rats were feeding better on bigger pieces of pellets from which they would break off smaller pieces to nibble at.

"Since rats are very similar to humans in terms of anatomy, physiology and genetics" (Maina, 2012) and much of the published information on the effect of aflatoxins in man has been obtained from the study of rats (Kensler et al., 2011), the negative correlation observed in this study can be extrapolated to human beings.

Exposure to aflatoxins results in alteration of intestinal function (Gong et al., 2008). As shown in Figure 10, most of the rats at post mortem had intestinal mucoid production which could have been caused by intestinal injury arising from consumption of aflatoxin contaminated feed. This injury could have led to less efficient food absorption and was probably the cause of the reduced food consumption which in turn led to a reduction in weight gain. This would account for the observed negative correlation between weight gain and consumption of aflatoxin contaminated food.

Previous research by Gong (2004) in Benin and Togo as well as research in Kisumu by Okoth and Ohingo (2004) validated the negative effects of aflatoxins on growth leading to malnutrition, thus supporting the findings of this study that demonstrate decrease in weight gain of rats fed on high and medium aflatoxin contaminated feed .

The fact that the regression analysis shows that length gain is not significantly correlated to aflatoxin levels could be accounted for by the brevity of the study. Stunting is a

product of chronic malnutrition and its detection would have required a longer study period.

Further analysis of the rat livers revealed a histopathological profile that agrees with literature on effect of aflatoxin exposure on rat livers (Wogan, 1966; Newberne & Butler, 1969). In this study, the livers of all the rats reacted to the treatment. Nuclear changes, cytoplasmic changes and fatty degeneration, all signs of cell injury and death were observed.

In the Wogan (1966) study, feeding of rats on partially purified aflatoxin feed was for 30 days. Incidence of tumours or lesions was observed 10 months after feeding on aflatoxin-free diets. The rats that were initially fed on 150 ppb had 100% incidence of cancerous tumours while those fed on 37.5 ppb and 15 ppb showed 100% and 80% incidence of precancerous lesions.

In this study, rats were fed for 21 days and post mortem and subsequent histopathological examination of the livers done immediately after. Though tumours or precancerous lesions were not observed as in the Wogan study above, the histopathological examination did detect liver injury in all the rats. It is probable that had the aflatoxin feeding been prolonged and post mortem and liver examination done after 10 months as in the Wogan study, the above liver injuries would have developed into cancerous tumours and lesions.

It is interesting to note that the rats fed on low aflatoxin levels of 6.90 ppb also reacted to the treatment and fatty liver changes and necrosis were evident. Though this level is below the KEBS aflatoxin limit of 10 ppb for total aflatoxins, the cumulative effect of 21 days exposure did result in liver damage. This indicates that chronic exposure of aflatoxins through porridge based foods can be detrimental to health even at low aflatoxin levels. Since children are often weaned on these foods and consumption of porridge continues even into adulthood for many people, the load of aflatoxin exposure to the disease burden in the country could be highly significant.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

MSMEs in Nairobi County supply a diversity of flour products ranging from cereal to legume flours with over 20 different flour products. These flours are often sold as composite flours to prepare porridge for both sick and healthy children and adults.

These flour products are contaminated with aflatoxins with groundnut and maize flours having the highest contamination levels. These enterprises receive already contaminated products from farm producers and middlemen and the quality control measures currently carried out by the MSMEs are inadequate to control the aflatoxin levels. At best, the role of these MSMEs in quality control is to prevent further contamination through proper storage of flour products.

The contaminated flour products supplied by MSMEs negatively affect weight gain and lead to liver damage and necrosis. Therefore, though porridges made from composite flours are indeed nutritious and in principal should lead to good health, the aflatoxin contamination in them counteracts their positive nutritional value and results in poor growth and liver damage. Efforts to combat aflatoxin contamination and exposure should therefore recognize the rising role these MSMESs are assuming along the supply chain of flour products.

The study also confirms that chronic exposure of aflatoxin levels below 10 ppb does lead to liver damage due to built up of aflatoxins in the serum.

6.2 RECOMMENDATIONS

The recommendations from this study are:

1. Public/consumer education campaign with two dimensions. On the one hand, enlightening people on the existence of aflatoxins and their negative health effects. On the other, educating on diet diversification to reduce intake of cereal flours that are most prone to aflatoxin contamination. The first awareness will sensitize and therefore push consumers to demand for safe and better quality flour products. The second would aim at behavioural change by providing alternatives to diet choices with the hope of reducing chronic exposure right from childhood (especially through weaning foods) to adulthood.
2. More research and subsequent consumer education on cheap, easy to implement, food based aflatoxin detoxifiers such as broccoli sprouts tea and green tea polyphenols. These would reduce the negative health impact of aflatoxin contamination.
3. Education of MSMEs on the health hazards of high levels of aflatoxin contamination in their flour products. Education on simple procedures such as flashing with water at high pressures and roasting of cereals as well as quality assurance protocols such as identification of clean sources of raw materials,

checking for sources of contamination and proper storage and packaging of products will significantly contribute to the reduction of aflatoxins.

4. Cereal farmers and flour millers could be encouraged to test their products using cheap and reliable aflatoxin testing kits and ensure that they are clear of aflatoxins. Such tested products could then be labeled as ‘safe from aflatoxins’. With increased public education and awareness of aflatoxins, it is hoped that consumers will be more willing to purchase these safe products at a slightly higher price. This in turn will push demand up for tested cereal and flour products.
5. Development of a policy by the Government to ensure quality and safety of flour supplied by MSMEs.

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APPENDIX I: QUESTIONNAIRE

SMALL AND MEDIUM ENTERPRISE MILLERS LOCATED WITHIN THE NAIROBI COUNTY

A. GENERAL INFORMATION

Questionnaire no: _____

1. Name of Interviewer: _____

3. Constituency: _____

4. Estate/Street: _____

Date: ___/____/____ Time Interview Started: _____
(Day/Month/Year)

B. ENTERPRISE INFORMATION

1. Name of Miller/Seller _____

Postal Address: _____

Tel No: _____

2. What is your core business?

1. Solely milling on contract or on order. (Therefore, I don't keep stock of the grains or flours)

2. Milling and selling flour products to customers/consumers.

3. Taking raw grains to millers for milling and then selling the milled products to customers/consumers.

4. Buying already milled flour and selling it to customers/consumers.

IF 2, 3 OR 4 PROCEED WITH THE QUESTIONNAIRE.

3. How many employees do you have? 1=1-9 2=10-19 3=20-29

4=30-39 5=40-49 6= 50 & >. **Specify**

4. How many days in a week do you operate your business? 1=1-2days 2=3-4days
3=5 days 4= 6-7 days 5= whenever there is work

5. On average, what is your daily sales? 1=<1 bag 2=1-3bags 3=4-6bags

4=7-9bags 5=10 and above bags. **Specify**

C. FLOUR PRODUCTS INFORMATION

1. Tick the pure flour products you supply/sell.

1. Maize meal
2. Maize flour (Heho/Unga baridi)
3. Sorghum (Mtama)
4. Finger millet (Wimbi)
5. Pearl millet (Uwele)
6. Cassava (Mhogo)
7. Wheat (Ngano)
8. Groundnuts (Njugu)
9. Silver cyprinid (Omena/dagaa)
10. Stinging nettle (Thabai=thafai)
11. Amaranth (Mchicha/terere)
12. Soya beans
13. Other beans or Pulses. ***Specify*:** _____

2. Do you also sell composite flours (mixes)? 1=Yes 2=No (Go to 3)

2.1. **If yes**, list the flour mixes that you supply and the **proportion** of each product in the mixture.

E.g. Sorghum (20g), Millet (10gm), Groundnuts (5 gms), Omena (3gms)

_____
_____
_____
_____
_____

3. Do customers consult on the appropriate flour types (either pure or mixes) to buy based on their needs?

1= Yes 2= No (If No, go to 5)

3.1. **If Yes**, how often

1= Consult but rarely
2= Often consult
3= Frequently consult

4. Based on the above consultation if any, what uses do customers have in mind when buying the milled products?

Usage	**Flour product**
1= to prepare porridge for normal children	_____
2= to prepare porridge for sick children.	
Specify disease: _____	_____
3= to prepare porridge for normal adults	_____

4= to prepare porridge for adults with diabetes _____
5 = to prepare porridge for adults with arthritis _____
6= others. ***Specify***: _____ _____
_____ _____

5. In terms of bags (90kgs), which milled products record the highest weekly sales? (To be written in order of highest to lowest. Indicate next to each the average daily sales in bags).

Pure flours	**Approximate weekly sales in bags.**
1. _____	_____
2. _____	_____
3. _____	_____
4. _____	_____
5. _____	_____
6. _____	_____
7. _____	_____
8. _____	_____

Flour Mixes	**Approximate weekly sales in bags**
1. _____	_____
2. _____	_____
3. _____	_____
4. _____	_____
5. _____	_____
6. _____	_____
7. _____	_____
8. _____	_____

6. Do you carry out quality control of the raw materials before milling? 1=Yes
2=No (Go to 7)

6.1. **If yes**, what kind of quality control is done, how is it done and why?

Test	How?	Why?
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

Cereal grains/product	**7. Where do you get your raw material(s) from?**	**7.1.0. If purchased, are you aware of the storage conditions of the raw materials before purchase?**	**7.1.1. If yes, what are some of the conditions you have encountered?**	**7.2.0 Do you clean the raw materials?**	**7.2.1 If yes, when do you clean?**
1. Maize					
2. Sorghum (Mtama)					
3. Finger millet (Wimbi)					
4. Pearl millet (Uwele)					
5. Cassava (Mhogo)					
6. Wheat (Ngano)					
7. Groundnuts (Njugu)					
8. Silver cyprinid (Omena/dagaa)					
9. Stinging nettle (Thabai=thafai)					
10. Amaranth (Mchicha/terere)					
11. Soya beans					
12. Other. Specify:					
	1=Own farm production. *Indicate location of farm. (Go to 7.2.0)* **2= I purchase. *From whom*? (Name of seller).**	**1=Yes.** **2=No (Go to 7.2.0)**		**1=Yes** **2=No (Go to 8)**	**1= Immediately after harvesting prior to milling** **2= Immediately after purchase prior to milling** **3= Immediately after harvesting prior to storage** **4=Immediately after purchase prior to storage** **5=any other time. *Specify***

Cereal grains/product		If yes to 8.0								
	8. Do you store your raw materials before milling?	**8.1. If yes, do you clean the raw materials before storage?**	**8.2. Do you dry the raw materials prior to storage?**	**8.3. Where do you store the raw materials?**	**8.4. In what packaging material are the raw materials stored?**	**8.5. 1. Do you monitor the conditions in the storage room/area ?**	**8.5.2. If yes, list the storage conditions in the room that you monitor?**	**8.6. For how long do you store your raw materials?**	**8.7.1. Do you clean your storage room and/or container**	**8.7.2. If yes, how often do you clean?**
1. Maize										
2. Sorghum (Mtama)										
3. Finger millet (Wimbi)										
4. Pearl millet (Uwele)										
5. Cassava (Mhogo)										
6. Wheat (Ngano)										
7. Groundnuts (Njugu)										
8. Silver cyprinid (Omena/dagaa)										
9. Stinging nettle (Thabai=thafai)										
10. Amaranth (Mchicha/terere)										
11. Soya beans										
12. Other. Specify:										
	1= Yes 2= No (Go to 9)	**1= Yes 2= No**	**1= Yes. *How?* 2= No**	**1= Separate special store 2=Inside the mill room 3=At home 4= Others. *Specify***	**1=Jute bag. 2= Sisal bag 3=Metallic container 4=Plastic bag 5=Plastic container 6=Placed on the floor 7= Others *Specify***	**1= Yes 2=No (Go to 8.6)**		**1=days-1week 2=2-3 wks 3=1 month 4=2-3 months 5=4 months and above**	**1= Yes 2= No (Go to 9)**	**1= Immediately after removing store content 2=2-6 days after removing store content 3= Other time. *Specify***

Milled flours/products	**9. Who does the milling of the raw materials?**	**9.1. Do you store your milled/purchased flour?**	**9.2. In what packaging material are the milled/purchased flours stored?**	**9.3. Where do you store the milled/purchased flours?**	**9.4.1. Do you monitor the conditions in the storage room/area?**	**9.4.2. If yes, list the storage conditions that you monitor?**	**9.5. For how long do you store your milled/purchased flours?**
1. Maize							
2. Sorghum (Mtama)							
3. Finger millet (Wimbi)							
4. Pearl millet (Uwele)							
5. Cassava (Mhogo)							
6. Wheat (Ngano)							
7. Groundnuts (Njugu)							
8. Silver cyprinid (Omena/dagaa)							
9. Stinging nettle (Thabai=thafai)							
10. Amaranth (Mchicha/terere)							
11. Soya beans							
12. Other. Specify:							
	1= Myself **2= I contract a miller. *Indicate name of miller*** **3=I buy already milled products. *From whom? (Name of seller)***	**1= Yes** **2= No. (End)**	**1=Jute bag.** **2= Sisal bag** **3=Metallic container** **4=Plastic bag** **5=Plastic container** **6=Placed on the floor** **7= Others. *Specify***	**1= Separate special store** **2=Inside the mill room** **3=At home** **4=At selling shop/kiosk** **5= Others. *Specify***	**1= Yes** **2= No (Go to 9.5)**		**1=days-1week** **2=2-3 weeks** **3=1 month** **4=2-3 months** **5=4 months and above**

APPENDIX II: CONSENT FORM

INTRODUCTION AND CONSENT

Hello. My name is _____. I am a student at the University of Nairobi doing nutrition. I am conducting a survey within the Nairobi County in order to obtain information on milled products supplied within the area. The survey hopes to establish the number of small and medium enterprise millers located within the county as well as the different types of flours that are frequently purchased by your customers. I would very much appreciate your participation in this survey.

The questionnaire will take between 15 to 30 minutes to complete.

Whatever information you provide will be kept confidential and will not be shared with anyone other than members of the survey team. The information will be used in writing up a Master's thesis with the hope that it will contribute to improving the nutritional status of the residents of Nairobi. No specific name of individual or milling company will be mentioned in the thesis.

Participation in this survey is voluntary. However, I hope that you will take part in it; your input is important.

At this time, do you want to ask me anything about the survey?

May I begin the interview now?

Respondent agreed to be interviewed _____ 1= Yes

2= No

Signature of interviewer _____

Date _____

APPENDIX III: RAT DATA USED IN GEE ANALYSIS

A) WEIGHTS OF RATS (GRAMS) TAKEN IN 7 VISITS OVER A PERIOD OF 21 DAYS

Aflatoxin Contamination Level	Sex	Rat No	WEIGHT MEASUREMENTS (g)						
			Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Low (6.90 ppb)	Females	1	85.83	106.28	118.43	125.81	140.56	144.91	154.34
		2	65.23	81.53	93.55	100.23	108.84	113.89	122.41
		3	61.52	73.87	84.55	92.80	103.21	105.72	114.70
	Males	1	71.99	88.91	98.98	108.40	120.97	128.66	139.90
		2	85.52	107.15	118.07	130.20	149.76	153.55	166.80
		3	74.96	89.89	100.76	111.05	123.43	127.77	141.02
Medium (45.51 ppb)	Females	1	65.89	82.48	90.55	100.67	112.41	116.98	125.67
		2	59.99	70.47	79.23	89.21	104.75	109.48	116.86
		3	67.25	81.40	93.37	103.30	121.92	129.68	138.29
	Males	1	49.94	62.99	70.54	78.96	89.94	94.94	103.45
		2	46.23	62.55	69.73	77.31	91.33	96.71	109.40
		3	45.43	60.33	67.06	77.41	91.10	100.16	106.72
High (148.45 ppb)	Females	1	52.72	55.40	63.09	72.58	88.06	92.52	101.41
		2	51.27	54.21	63.39	72.89	91.08	95.84	101.80
		3	48.45	54.52	62.99	73.86	88.91	94.45	102.09
	Males	1	49.46	60.46	69.37	74.62	90.30	99.06	107.98
		2	63.09	80.55	89.51	101.65	123.10	132.01	143.98
		3	64.88	78.93	86.84	96.98	112.66	122.15	130.82

B) LENGTH OF RATS (CM) TAKEN IN 7 VISITS OVER A PERIOD OF 21 DAYS

Aflatoxin Contamination Level	Sex	Rat No.	LENGTH MEASUREMENTS (cm)						
			Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Low (6.90 ppb)	Females	1	25	26.5	28	28.5	30.5	31.5	32.1
		2	23	24	26	27.5	28.5	29.2	30.8
		3	23	25.5	25.5	26.2	27.5	28.1	30.1
	Males	1	24.5	25	27	27.2	28.2	29	30.3
		2	26	26	26.5	27	31.6	33.6	34.3
		3	24	24	25	27.5	30.5	31.2	32
Medium (45.51 ppb)	Females	1	22	23.7	24.8	25.3	27.3	28.2	29.5
		2	23	25.4	26.1	26.4	29.2	30.7	31.7
		3	24	26.1	27	28.7	30.3	30.7	32.8
	Males	1	22	23.9	24.7	25.5	27	28.3	30.2
		2	21	22.7	23.7	24.9	27	27.8	28.9
		3	21	22.3	23.3	25.4	27	29	30.1
High (148.45 ppb)	Females	1	22	22	22	24.5	27.8	28.9	29.6
		2	22	22	22	24.5	27.3	28.5	29.8
		3	22	22.4	22.5	24.4	27.1	28	29.3
	Males	1	20.5	22	23	24.5	27.1	28.5	30.7
		2	24	24	26	26.5	30.3	31.3	32
		3	23.5	24	25.5	26	29.8	30.9	31.5

C) FOOD CONSUMPTION (GRAMS) WEIGHED IN 7 VISITS OVER A PERIOD OF 21 DAYS

Aflatoxin Contamination Level	Sex	FOOD CONSUMPTION PER RAT(g)						
		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Low (6.90 ppb)	Females	13.12	17.94	16.28	15.43	14.10	14.02	13.99
	Males	16.72	18.25	17.05	16.97	18.13	16.89	18.26
Medium (45.51 ppb)	Females	5.09	15.29	12.60	13.46	12.84	13.69	
	Males	4.31	8.18	10.63	11.21	11.95	15.55	
High (148.45 ppb)	Females	6.48	11.99	12.83	14.61	14.67	13.92	11.84
	Males	13.07	14.05	13.11	15.22	16.60	17.01	16.40

APPENDIX IV: GENERALIZED ESTIMATING EQUATIONS REGRESSION ANALYSIS OUTPUT

TAKING WGTGAIN AS THE RESPONSE VARIABLE

Regression analysis

Response variate: workvar
Weight variate: wgtgain
Fitted terms: Constant + Sex + Afla_ppb + Length_cm + Consumption_g

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.
Regression	5	14538.	2907.6	11.03
Residual	114	30046.	263.6	
Total	119	44584.	374.7	

Percentage variance accounted for 29.7
Standard error of observations is estimated to be 16.2.

Estimates of parameters

Parameter	estimate	s.e.	t(114)
Constant	113.2	14.3	7.92
Sex Male	5.06	3.07	1.65
Afla_ppb 2	-19.51	4.71	-4.14
Afla_ppb 3	-13.91	3.84	-3.62
Length_cm	-1.845	0.580	-3.18
Consumption_g	-2.739	0.672	-4.07

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Sex	Female
Afla_ppb	1

Correlations between parameter estimates

Parameter	ref	correlations					
Constant	1	1.000					
Sex Male	2	-0.022	1.000				
Afla_ppb 2	3	-0.401	-0.157	1.000			
Afla_ppb 3	4	-0.394	-0.086	0.575	1.000		
Length_cm	5	-0.749	0.098	-0.106	0.052	1.000	
Consumption_g	6	-0.161	-0.255	0.581	0.301	-0.510	1.000
		1	2	3	4	5	6

Autoregressive correlation structure.

Scale factor 334.8

Matrix of correlations

1	1.0000				
2	0.5369	1.0000			
3	0.2882	0.5369	1.0000		
4	0.1547	0.2882	0.5369	1.0000	
5	0.0831	0.1547	0.2882	0.5369	1.0000
6	0.0446	0.0831	0.1547	0.2882	0.5369
7	0.0239	0.0446	0.0831	0.1547	0.2882
	1	2	3	4	5

6	1.0000		
7	0.5369	1.0000	
	6	7	

Model estimates of s.e.

	Estimate	s.e.
Constant	168.06	16.116
Sex Male	8.16	4.889
Afla_ppb 2	-27.63	6.968
Afla_ppb 3	-18.80	6.364
Length_cm	-3.17	0.610
Consumption_g	-3.89	0.643

Correlations

1	1.0000				
2	-0.1476	1.0000			
3	-0.4562	-0.0792	1.0000		
4	-0.3694	0.0054	0.5151	1.0000	
5	-0.7904	0.1468	0.0331	0.0787	1.0000
6	-0.0731	-0.2612	0.4268	0.1615	-0.4922
	1	2	3	4	5

6	1.0000	
	6	

Sandwich estimates of s.e.

	Estimate	s.e.
Constant	168.06	15.324
Sex Male	8.16	4.730
Afla_ppb 2	-27.63	5.920
Afla_ppb 3	-18.80	4.866
Length_cm	-3.17	0.554
Consumption_g	-3.89	0.422

Correlations

1	1.0000				
2	0.0936	1.0000			
3	-0.3587	0.0873	1.0000		
4	-0.3928	0.2579	0.4944	1.0000	
5	-0.8474	-0.1835	0.1495	-0.0421	1.0000
6	-0.1695	-0.3384	0.1524	0.5397	-0.2896
	1	2	3	4	5

6	1.0000
	6

TAKING LENGTH GAIN AS THE RESPONSE VARIABLE

Regression analysis

Response variate: workvar
Weight variate: Length
Fitted terms: Constant + Sex + Afla_ppb + Wgtgain + Consumption_g

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.
Regression	5	446.3	89.258	14.13
Residual	114	720.0	6.316	
Total	119	1166.3	9.801	

Percentage variance accounted for 35.6
Standard error of observations is estimated to be 2.51.

Estimates of parameters

Parameter	estimate	s.e.	t(114)
Constant	21.96	1.83	11.98
Sex Male	-0.250	0.480	-0.52
Afla_ppb 2	-0.072	0.782	-0.09
Afla_ppb 3	-0.932	0.621	-1.50
Wgtgain	-0.0442	0.0139	-3.18
Consumption_g	0.422	0.104	4.05

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Sex	Female
Afla_ppb	1

Correlations between parameter estimates

Parameter	ref	correlations					
Constant	1	1.000					
Sex Male	2	0.165	1.000				
Afla_ppb 2	3	-0.765	-0.201	1.000			
Afla_ppb 3	4	-0.587	-0.138	0.629	1.000		
Wgtgain	5	-0.600	-0.174	0.375	0.297	1.000	
Consumption_g	6	-0.961	-0.292	0.683	0.464	0.511	1.000
		1	2	3	4	5	6

Autoregressive correlation structure.

Scale factor 8.009

Matrix of correlations

1	1.0000				
2	0.7171	1.0000			
3	0.5142	0.7171	1.0000		
4	0.3687	0.5142	0.7171	1.0000	
5	0.2644	0.3687	0.5142	0.7171	1.0000
6	0.1896	0.2644	0.3687	0.5142	0.7171
7	0.1360	0.1896	0.2644	0.3687	0.5142
	1	2	3	4	5

6	1.0000	
7	0.7171	1.0000
	6	7

Model estimates of s.e.

	Estimate	s.e.
Constant	28.404	1.7183
Sex Male	-0.873	0.7658
Afla_ppb 2	-2.929	1.1449
Afla_ppb 3	-2.154	1.1163
Wgtgain	-0.059	0.0099
Consumption_g	0.148	0.0915

Correlations

1	1.0000				
2	0.0174	1.0000			
3	-0.7191	-0.0931	1.0000		
4	-0.5093	0.0139	0.5187	1.0000	
5	-0.6191	-0.1919	0.2540	0.1578	1.0000
6	-0.8692	-0.2767	0.5329	0.2376	0.6433
	1	2	3	4	5

6	1.0000	
	6	

Sandwich estimates of s.e.

	Estimate	s.e.
Constant	28.404	1.6453
Sex Male	-0.873	0.9186
Afla_ppb 2	-2.929	0.9403
Afla_ppb 3	-2.154	0.9097
Wgtgain	-0.059	0.0102
Consumption_g	0.148	0.0951

Correlations

1	1.0000				
2	0.0545	1.0000			
3	-0.6684	-0.3831	1.0000		
4	-0.5717	-0.0088	0.6487	1.0000	
5	-0.7453	-0.1881	0.3784	0.1107	1.0000
6	-0.9308	-0.2849	0.5689	0.4227	0.8021
	1	2	3	4	5

6	1.0000
	6

TAKING FOOD CONSUMPTION AS THE RESPONSE VARIABLE

Regression analysis

Response variate: workvar
Weight variate: Consumption
Fitted terms: Constant + Sex + Afla_ppb + Wgtgain + Length_cm

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.
Regression	5	811.5	162.293	36.35
Residual	114	509.0	4.465	
Total	119	1320.5	11.096	

Percentage variance accounted for 59.8
Standard error of observations is estimated to be 2.11.

Estimates of parameters

Parameter	estimate	s.e.	t(114)
Constant	8.24	2.18	3.77
Sex Male	1.253	0.387	3.24
Afla_ppb 2	-4.461	0.508	-8.79
Afla_ppb 3	-2.144	0.488	-4.40
Wgtgain	-0.0464	0.0114	-4.07
Length_cm	0.2981	0.0736	4.05

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Sex	Female
Afla_ppb	1

Correlations between parameter estimates

Parameter	ref	correlations					
Constant	1	1.000					
Sex Male	2	-0.026	1.000				
Afla_ppb 2	3	-0.417	-0.021	1.000			
Afla_ppb 3	4	-0.417	-0.021	0.535	1.000		
Wgtgain	5	-0.541	-0.055	0.187	0.215	1.000	
Length_cm	6	-0.981	-0.061	0.324	0.317	0.472	1.000
		1	2	3	4	5	6

Autoregressive correlation structure.

Scale factor 5.033

Matrix of correlations

1	1.0000				
2	0.3754	1.0000			
3	0.1409	0.3754	1.0000		
4	0.0529	0.1409	0.3754	1.0000	
5	0.0199	0.0529	0.1409	0.3754	1.0000
6	0.0075	0.0199	0.0529	0.1409	0.3754
7	0.0028	0.0075	0.0199	0.0529	0.1409
	1	2	3	4	5

6	1.0000	
7	0.3754	1.0000
	6	7

Model estimates of s.e.

	Estimate	s.e.
Constant	8.579	2.4174
Sex Male	1.768	0.5170
Afla_ppb 2	-4.101	0.6669
Afla_ppb 3	-2.134	0.6923
Wgtgain	-0.054	0.0110
Length_cm	0.274	0.0788

Correlations

1	1.0000				
2	-0.1017	1.0000			
3	-0.3340	0.0055	1.0000		
4	-0.3921	0.0319	0.5251	1.0000	
5	-0.6266	-0.0252	0.1516	0.1775	1.0000
6	-0.9731	-0.0056	0.2043	0.2643	0.5901
	1	2	3	4	5

6	1.0000	
	6	

Sandwich estimates of s.e.

	Estimate	s.e.
Constant	8.579	2.6606
Sex Male	1.768	0.4773
Afla_ppb 2	-4.101	0.3601
Afla_ppb 3	-2.134	0.2625
Wgtgain	-0.054	0.0136
Length_cm	0.274	0.0955

Correlations

1	1.0000				
2	0.3947	1.0000			
3	0.6494	0.2346	1.0000		
4	-0.8036	-0.1661	-0.4729	1.0000	
5	-0.3491	0.3735	-0.5131	0.4273	1.0000
6	-0.9882	-0.5146	-0.6096	0.7540	0.2236
	1	2	3	4	5

6	1.0000
	6