DETOXIFICATION OF AFLATOXINS IN FEED INGREDIENTS FOR BROILER CHICKEN FEED THROUGH FERMENTATION

ISABELLE BEATRICE MUKANDUNGUTSE

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted or presented in any other institution.

Signature……. ……Date…….....

Ms. Isabelle B. Mukandungutse

KM113/12089/17

Recommendation

This thesis has been submitted with our approval as university supervisors.

Signature……. ……Date…….....

Prof. James K. Tuitoek

Department of Animal Sciences

Egerton University

Signature……. ……Date…….....

Dr. Anthony M. King'ori

Department of Animal Sciences

Egerton University

Signature……. ……Date…….....

Dr. Meshack A. Obonyo

Department of Biochemistry & Molecular Biology

Egerton University

DEDICATION

I hereby wish to dedicate this work to my husband Deogratias Mazimpaka and our son Odhran Genie Shimwa, I love you all.

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ABSTRACT

Aflatoxins (AFs) are some of the toxic and carcinogenic mycotoxins. Due to the high cost of most cereals and its competition for human food, chicken feeds are often formulated with mouldy and broken grains which are easily contaminated with mycotoxins. Kenya Bureau of Standards recommended 20ppb total AFs as the permissible level in poultry feed. The first objective of this study evaluated the effects of fermentation on the detoxification of AF contaminated maize flour using *S. cerevisiae*. This was achieved in 3 experiments where the first and second experiments, maize flour were fermented with and without *S. cerevisiae* for 5-8 days. In the third experiment, the flour to water ratio was adjusted from 1:1 to 2:3 and fermented for 72 hours. Fermentation reduced pH (from 6.9 to 5.0) and total AFs by 52 and 53.4% when fermented either with or with *S. cerevisiae* respectively. It is concluded that the best ratio of flour to water for fermentation was 2:3 and the fermentation period of 72 hours. The second objective evaluated the effect of fermented feed on the digestibility of dry matter, metabolizable energy content, and nitrogen utilisation in broiler chicken. Twenty-four, 28-day old male broiler chicken were assigned to six different dietary treatments which are: diet 1 (no AF and not fermented), diet 2 (no AF and fermented without *S. cerevisiae*), diet 3 (no AF and fermented with *S. cerevisiae*), diet 4 (contained 20.034ppb AF and not fermented), diet 5 (contained 20.034ppb AF and fermented without *S. cerevisiae*) and diet 6 (contained 20.034ppb AF and fermented with *S. cerevisiae*). Each diet was assigned to four chicken following a 7-day adaptation period and 7-day total faecal collection period. The results showed that dry matter digestibility and metabolizable energy were significantly ($p<0.05$) affected by fermentation types. The metabolizable energy and nitrogen were significantly ($p<0.05$) affected by the total AF level of 20.034ppb in the diet. The third objective evaluated the feed intake and growth of broiler chicken offered fermented feed with or without *S. cerevisiae*. One hundred and forty-four broiler chicks were fed six different diets for 21 days. The six diets were: diet I (no AF and unfermented), diet II (no AF and fermented without *S. cerevisiae*), diet III (no AF and fermented with *S. cerevisiae*), diet IV (30.08ppb AF and not fermented), diet V (30.08ppb AF and fermented without *S. cerevisiae*) and diet VI (30.08ppb AF and fermented with *S. cerevisiae*). The intake, growth, and gain: feed ratio was calculated. Mortality was recorded every day. Results showed no significant difference of six diets on intake and growth. However, gain: feed ratio was significantly ($p=0.048$) better in broilers fed diets which were fermented without yeast. The mortality rate was very high (75.0%) in chicks fed on the diet containing 30.08ppb, which was not fermented.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFB1	Aflatoxin B1
AFB2	Aflatoxin B1
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFs	Aflatoxins
CESAAM	Centre of Excellence in Sustainable Agriculture and Agribusiness Management
CP	Crude Protein
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organization of the United Nation
FM	Fish Meal
GDP	Gross Domestic Product
KES	Kenya Shillings
LAB	Lactic Acid Bacteria
LC/MS	Liquid Chromatography/ Mass Spectrometry
ME	Metabolizable Energy
NRC	National Research Council
ppb	parts per billion
SAS	Statistical Analysis System
SBM	Soybean Meal

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Aflatoxins (AFs) are some of the most toxic and carcinogenic mycotoxins produced by some species of filamentous fungi like *Aspergillus parasiticus* and *Aspergillus flavus* (Sineque *et al.*, 2017). *Aspergillus parasiticus* and *flavus* are the primary contaminants of common feed ingredients used in poultry feeds. The fungi are found living freely in soils of many countries, especially in tropical and subtropical regions, where temperature and humidity are optimal for the growth of moulds and the production of toxins (Suhaimi *et al.*, 2017). Moisture and temperature play a significant role in fungal growth and the production of aflatoxins. Mycotoxin-producing fungi frequently need higher moisture levels (20–25%) for infection during the pre-harvest phase in the field than fungi that proliferate during storage (13–18%) (Bryden, 2012). *Aspergillus parasiticus* and *Aspergillus flavus* grow well at 25–35^{o}C, when spores encounter suitable nutrients like cereals (maize, groundnuts) and favourable environmental conditions like (pH, moisture, presence of O_2, CO_2 gases); the fungus rapidly colonises and successfully produces aflatoxins (Lakkireddy *et al.*, 2014). Mycotoxins of importance are often found as natural contaminants in raw ingredients of poultry feed (Khan *et al.*, 2011). Among the 400 known mycotoxins, aflatoxins B1 (AFB1), aflatoxins B2 (AFB2), aflatoxins G1 (AFG1) and aflatoxins G2 (AFG2) are the most significant in foods and feeds due to their negative effect on human health (Jalili, 2016).

It is estimated that 25% of the world's food crops are affected by mycotoxins and according to FAO estimates, global losses of foodstuffs due to mycotoxins are in the range of 1000 million tonnes per year (Iheshiulor et al., 2011). Aflatoxins are not only a big problem at crop production level but have also become a global health issue because of the consequences that their consumption generates in animals and human beings (Lizárraga-Paulín *et al.*, 2011). Poultry are among the most sensitive livestock to the toxic and carcinogenic action of AFB1, resulting in annual losses estimated in millions of dollars (Rawal *et al.*, 2010). In chicken, aflatoxins impair most of the essential production parameters including weight gain, feed intake, feed conversion efficiency, pigmentation, processing yield, egg production, and male and female reproductive performance (Hussain *et al.*, 2010).

According to many regulatory bodies on aflatoxins, tolerable levels of total aflatoxins in animal feeds are different. The World Health Organization (WHO) set aflatoxins limits of 5ppb for animals (Kajuna *et al*., 2013) while United States Food and Drug Administration and European Commission have set 20ppb as the worldwide range of maximum tolerable level of total aflatoxins for poultry feed (Syahidah *et al.,* 2017). In 2003, Kenya was one of only five African countries to report standards on aflatoxins. Kenyan standards likely match East African Standards which specify maximum aflatoxin limits as 20ppb and 10ppb AFB1 for adult and young poultry feed, respectively (Sirma *et al.,* 2018).

Detoxification strategies for contaminated foods and feeds should be done to reduce or eliminate the adverse actions of mycotoxin to improve food safety and prevent economic losses (Zaki *et al*., 2012). They are different methods for aflatoxins detoxification like physical, chemical and biological methods. Although chemical and physical methods used at present are, to some extent, successful, they have some disadvantages like limited efficacy, possible loss of essential nutrients, release mycotoxins from masked forms and make them bioavailable or convert mycotoxins into forms not detectable by conventional analytical methods, and frequently high costs (Rychlik *et al.,* 2014). Based on these disadvantages of the physical and chemical methods, biological methods are the means of controlling aflatoxins to protect the quality of food or feed because they are considered safer (Gonçalves *et al*., 2017).

Many workers in the field opine that the best solution for decontamination should be detoxification by biodegradation, giving a possibility for removal of mycotoxins under mild conditions without affecting the quality of feed (Sezer *et al.,* 2013). Fermentation, one of the biological methods, is the easiest and cheapest means of feed preservation in addition to imparting nutritional and organoleptic benefits to fermented feed through the use of yeast or bacteria (Juodeikiene *et al.,* 2012; Wang *et al.,* 2018). *Saccharomyces cerevisiae*, the most well-known species of yeast, is used to carry out fermentation and represents also the high potential application in reducing levels of aflatoxins by binding metabolites of filamentous fungi to the components of the cell wall called oligomannanes after their chemical modification and esterification (Gonçalves *et al.,* 2017; Joannis-Cassan *et al.,* 2011). It is therefore desirable to ferment feed ingredients to remove the harmful effects of AFs in chicken feed.

1.2 Statement of the Problem

Poultry feeds are usually formulated using cereals, their by-products and oil cakes to supply the required nutrients to chicken. Due to the high cost of poultry feed ingredients and competition for human food, chicken feeds are often formulated from ingredients that are unsuitable for humans consumption (mainly mouldy broken grains, cereal, and oilseed processing by-products). These ingredients are contaminated with mycotoxins, the most common among them being aflatoxins. Consumption of aflatoxin-contaminated poultry feed results in depressed performance, death of poultry and contamination of poultry products (meat and eggs) which end up in the human food chain. The exposure of aflatoxins in humans has been associated with liver cancers and a lowered immunity to diseases. Several decontamination procedures such as physical and chemical methods have been proposed and tested to mitigate and reduce human exposure to aflatoxins but none has so far been conclusively effective or practically feasible for a variety of reasons including cost considerations.

1.3 Objectives

1.3.1 General objective

The overall objective of this study was to contribute to food safety and quality through the reduction of aflatoxins in chicken meat.

1.3.2 Specific objectives

i. To determine the effect of the period (hours) of fermentation using *S. cerevisiae* on the concentration of aflatoxin in maize.
ii. To determine the effect of fermentation of broiler feed using *S. cerevisiae* on dry matter digestibility, metabolizable energy, and nitrogen content in broiler chicken.
iii. To determine the effect of fermentation of broiler feed using *S. cerevisiae* on feed intake and growth of broiler chicken.

1.4 Hypotheses

i. Fermentation periods using *S. cerevisiae* does not affect the concentration of aflatoxins in contaminated maize.
ii. Fermentation of broiler feed using *S. cerevisiae* has no effect on dry matter digestibility, metabolizable energy, and nitrogen content in broiler chicken.
iii. Fermentation of broiler feed using *S. cerevisiae* does not affect feed intake and growth of broiler chicken.

1.5 Justification

Aflatoxin contamination of feeds and food material has a significant impact on human health and the trade of agricultural commodities. Aflatoxins were found to be prevalent (>20ppb) in commercial, broiler starter and broiler finisher feeds in Nakuru-Kenya, and the levels of the toxins exceeded the maximum permissible limits for poultry feed (Thuita *et al*., 2019). Many approaches of detoxification of aflatoxins such as physical methods, chemical methods, and biological methods have been tested and used, but these methods have various limitations. Physical processes are time-consuming, while chemical methods lower the aesthetic quality of feed and come with attendant high costs. Based on these limitations, biological methods are the most promising alternative to detoxify AFs from the feed material. Fermentation, a biological method, offers some advantages such as product specificity, mild reaction conditions, and feasible processes in food and feed industries. It is an easy and cheap form of feed processing and preservation method that, also, imparts nutritional and organoleptic benefits to fermented feeds. *In vitro* studies suggest that fermentation by *S. cerevisiae* is a better method of detoxification of aflatoxins at a low cost. This study, therefore, evaluated the effect of fermentation in detoxifying aflatoxin in contaminated poultry feed ingredients.

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CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of Broiler Chicken Production in Kenya

In Kenya, agriculture contributes 26% of the country's gross domestic product (GDP), while poultry production represents roughly a third (30%) of the agricultural GDP (FAO, 2008). Broilers constitute about 60% of the total commercial birds produced (De Groote *et al.,* 2010). There are reports that around 2012, broiler chickens were 1.6 times (409,715) the numbers of indigenous chickens (261,773) consumed in Nairobi County (GoK, 2012).

The consumption of poultry meat in Kenya is predicted to increase from 54.8 thousand metric tons (TMT) in 2000 to 164.6 (TMT) by the year 2030 (Carron *et al.*, 2017). The increase in Nairobi is from 6 to 30.5 TMT due to urbanisation, population growth, economic growth making people wealthier, and the continuing viability of current broiler chicken systems (Carron *et al.,* 2017). To meet the expected growth in demand, poultry production in Kenya is anticipated to increase from 56.9 to 1666 metric tons by 2030 (FAO, 2008). The assumption is that trade regulations remain similar and relative prices of inputs and outputs to the poultry system remain unchanged (Carron *et al.*, 2017). In the poultry sector, the data indicates a change from indigenous breed chicken meat production to an intensive broiler production system that needs concentrate feeds (Narrod *et al*., 2008).

2.2 Occurrence of Aflatoxins in Animal Feeds

The conditions under which fungi contaminate feed material and eventually produce mycotoxins in agricultural commodities highly depend on environmental factors, primarily moisture level and temperature. Similarly, slightly elevated carbon dioxide concentration may stimulate the growth of mycotoxigenic fungi (Magan *et al*., 2011). Additionally, plant stress due to extreme weather situations, such as drought, results in plants becoming more vulnerable to fungal infection (Wu *et al*., 2011).

In sub-Saharan Africa, aflatoxin contamination of food material quite prevalent, as reported in many countries. For example, in central Tanzania, it was found that staple crops, maize, sorghum, Bambara nuts, groundnut, and sunflower common in semi-arid agro-pastoral farming systems are prone to aflatoxin contamination which affects plant growth and health (Seetha *et al.,* 2017). In Rwanda, mycotoxins analyzed in sample maize flour were found to be 89%, 67%, 78% and 33% for AFB1, AFB2, AFG1, and AFG2 respectively. The highest aflatoxin concentration was 16.8 and 126.6ppb in maize and peanut flour, respectively

(Umereweneza *et al.*, 2018). This high concentration is favoured by the average temperature, which varies between 14 and 30^{o}C and relative humidity between 71% and 79% (Matsiko *et al.*, 2016). Nishimwe *et al.* (2017) found that 75% of animal feed samples have more than 100ppb of aflatoxin B1 in Rwanda. In Uganda, aflatoxin levels in the food samples ranged from 0 to 55ppb (Kitya *et al*., 2010). In Nigeria, it was reported that 33% of maize samples from different agro-ecological zones are contaminated with Aflatoxins (Udoh *et al*., 2000).

In South Africa, cattle feeds were probably more contaminated with mycotoxins than feeds for other animal species. They were found to contain the highest (>27.5ppb) mean levels of aflatoxins (Njobeh *et al*., 2012). Aflatoxins in South Africa are among important mycotoxins that contaminate field crops (groundnut, maize, cottonseed, and rice) that significantly impact human and animal productivity (Ncube, 2008).

The occurrence of aflatoxin in Malawi is not crop-specific as evidenced by the presence in the two target crops, maize and groundnuts (Waliyar *et al*., 2013). Aflatoxins are found in a wide range of crop species, including cereals and legumes. Its contamination ranges from 0.0 to 3871ppb in samples collected from which country (Waliyar *et al*., 2013). In Zimbabwe, levels of Aflatoxin B_1 ranged between 0.57 and 26.6ppb in maize samples, which is 21% of aflatoxin B1 contamination (Murashiki *et al*., 2017). In Egypt, mycotoxins are common in foods and are a concern for public health. The highest content of AFB1 is in nuts and seeds, which was 82% (Darwish *et al*., 2014).

2.3 Metabolism of Aflatoxins in Chicken

Understanding the metabolic pathways of mycotoxins could enable researchers and public health officials to gain insights on how to assess the associated risks of mycotoxin exposure in various species (Husein and Brasel, 2001). The metabolism of aflatoxins involves oxidative reactions. After AFB1 ingestion and transportation to the liver, there is enzymatic metabolism by the cytochromes P450 (CYP450) of hepatocytes to form its primary carcinogenic metabolites AFB1-8,9-exo-epoxide and AFB1-8,9-endo-epoxide (AFBO), or to less mutagenic forms such as aflatoxin M1 (AFM1), aflatoxin P1 (AFP1) and aflatoxin Q1 (AFQ1) (Groopman *et al*., 2008; Diaz *et al.*, 2010). They are two phases of aflatoxin metabolism. The first phase converts original molecules into more hydrophilic compounds utilising mainly enzymatic hydrolytic and oxidative–reduction reactions. The second phase is characterized by conjugation of the original molecule or its metabolites with nucleophilic molecules such as glutathione and glucuronides (Dohnal *et al*., 2014).

Aflatoxin B1 absorbed into the organism could be metabolized significantly in four metabolic pathways: O-dealkylation to AFP1, ketoreduction to aflatoxicol (AFL), epoxidation to AFB1-8,9-epoxide (acutely toxic, mutagenic and carcinogenic) and hydroxylation to AFM, AFP1, AFQ1 or AFB2a (Wu *et al.*, 2011). AFB1-8,9-exo-epoxide, the activated AFB1 is highly unstable, and readily binds to cellular macromolecules such as proteins and DNA, to form 8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB1 (AFB1–N7-Gua) adduct (Groopman and Kensler, 2005) and results in G→T transversions, DNA repair, lesions, mutations and subsequently tumour formation (Husein and Brasel, 2001). AFB1 may be degraded to some new products whose structural properties are different from AFB1, or they dissolve in the aqueous phase rather than in the organic one (Zhou *et al*., 2017).

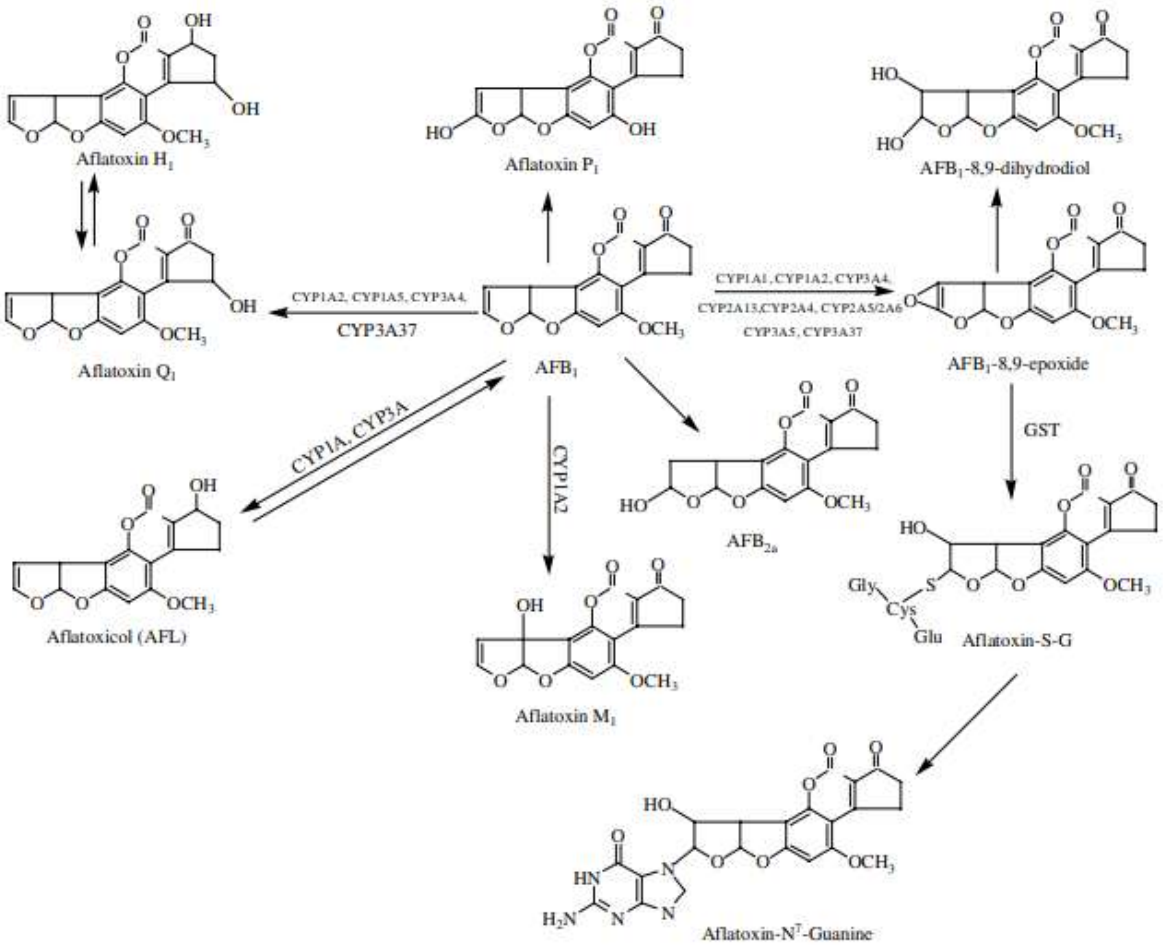

Figure 2.1 The major metabolic pathway.0ys and critical metabolizing enzymes of Aflatoxin B1 in animals and human beings (Dohnal *et al.*, 2014).

2.4 Effects of Aflatoxins on Animal Performance and the Food Chain

Contamination by aflatoxin can take place at any point along the food or feed chain from the field, harvest, handling, shipment, and storage under a wide range of climatic conditions. When aflatoxin-contaminated feed is consumed by poultry, important production parameters, including weight gain, feed intake, feed conversion efficiency, and reproductive performance, are also compromised (Suhaimi *et al*., 2017).

Aflatoxicosis in poultry also causes changes in biochemical and haematological parameters, liver and kidney abnormalities, and impaired immunity, which may enhance susceptibility to infectious diseases (Fareed *et al*., 2014). In pigs, the first sign of aflatoxin problem is decreased feed intake and depending on the levels present; losses can result from deaths, reduced growth rates, poor feed conversion efficiency, and carcass condemnations (Iheshiulor *et al*., 2011). Aflatoxins have been shown to negatively affect production, immune system function, and rumen metabolism in cattle. Aflatoxins also affect the quality and quantity of milk produced by dairy cows (Zain, 2011). In lambs, aflatoxin exposure resulted in low average daily gain and altered the immune response, which could render the animals more susceptible to infectious diseases (Fernandez *et al*., 2000).

2.5 Management of Aflatoxins in Contaminated Feed

The efficient reduction of mycotoxin exposure via food products requires the integrated approach of all available technologies from good agricultural and storage practices and the selection of raw materials suitable for human consumption to the application of food processing technologies (Karlovsky *et al.*, 2016). Different detoxification strategies of feeds should be done to reduce or eliminate the adverse effects of aflatoxins on feed and to improve the safety of the food. The process of detoxification of aflatoxins can be done by physical, chemical and biological methods.

2.5.1 Physical methods

The physical control strategies for aflatoxins can be divided into good agricultural practices (pre-harvest strategies) and good storage practices (post-harvest strategies). Pre-harvest strategies include the use of genetically improved crops that are resistant to *Aspergillus* infection and environmental stressors, utilization of pesticide, crop rotation, land tillage and timing of planting. Post-harvest strategies include proper drying, packaging, storage, and preservatives/pesticide usage. Other physical methods are irradiation and heat treatments. Aflatoxins are well known to be stable at high temperatures, so harsh heating is

needed to effectively remove amounts. Recent studies have shown that temperatures of 150-200^{o}C can remove significant amounts of AFB1 (an average of 79% reduction), which is most effective at high humidity (Arzandeh and Jinap, 2011; Lee *et al*., 2015). One of the challenges of this strategy is ensuring the integrity of the product after the heating/roasting is complete. This sometimes limits the maximum temperature that can be used, which may result in only partial removal of AFB1 (Rushing and Selim, 2019).

2.5.2 Chemical methods

The use of chemicals on contaminated ingredients has also become a popular choice particularly if the chemicals themselves are already used in the food and feed industry. Some of the chemical treatments are acidification (treatment by acids), ammoniation (treatment by ammonia) and Ozonation. Acidification of AFB1 contaminated ingredients has been shown to be highly effective when citric, lactic, tartaric, and hydrochloric acid are used, however other acids such as succinic, acetic, ascorbic, and formic have only been marginally successful (Rushing and Selim, 2019).

2.5.3. Biological methods

Biological decontamination methods are the means of controlling aflatoxins to protect the quality of food or feed because they are considered safer (Gonçalves *et al*., 2017). Studies using biotechnology to reduce AFB1 levels in contaminated ingredients use plant extracts to degrade AFB1 (Su, 2019), and other inoculate microorganisms (bacteria and yeasts), extracts (*Trachyspermum ammi, Corymbia citriodora*) and purified enzymes (laccase, manganese peroxidase, Bacillus aflatoxin-degrading enzyme) (Iram *et al.,* 2016; Xu *et al.,* 2017). Some of the bacteria and yeasts used to detoxify aflatoxins are *Lactobacillus plantarum, Lactobacillus acidophilus*, *Saccharomyces cerevisiae* and *Kluyveromyces lactis* (Hamad *et al.,* 2017).

Microorganism shows promise as a better alternative to AFs decontamination as it involves the use of microbial catabolic pathways to detoxify the AFs to less toxic intermediates or end products (Samuel *et al*., 2013) and offers some advantages such as product specificity, mild reaction conditions, and feasible processes when applied in food and feed industries (Kolosova and Stroka, 2011). Among the different decontaminating microorganisms, yeast represents a unique group and is one of the well-known strategies for its management in foods and feeds **(**Deepak *et al.,* 2015). Yeast has been known for ages to carry out fermentation in food processing and preservation (Hathout and Aly, 2014).

Fermentation, being one of the oldest forms of food processing and preservation in the world is the easiest and cheapest means of food preservation in addition to imparting nutritional and organoleptic benefits to fermented foods (Juodeikiene *et al.,* 2012). Fermentation is effected by natural microbiota of raw materials, microorganisms attached to the fermentation equipment, or from externally added starter cultures (Biernasiak *et al.,* 2006). Fermentation of feed, using specific fungi or bacteria can degrade antinutritional compounds to improve the nutrient uptake in feed for domestic animals. It can also provide probiotics and their metabolites and can be a possible alternative to growth-promoting antibiotics (Wang *et al.,* 2018). Yeasts have immense potential as tools in tackling the problem of mycotoxins in cereal-based foods and animal feeds (Juodeikiene *et al*., 2012). It has been reported that yeast, *S. cerevisiae,* can be included in the broiler diet and could, therefore, serve as a natural substitute for antibiotics (Ahmed *et al*., 2015). The addition of 0.1 or 0.2% of the powdery form of live yeast *S. cerevisiae* to diet improve humoral immune responses, decrease serum lipids and suppress abdominal fat accumulation in broiler chickens (Gheisari and Kholeghipour, 2006).

2.6 Mode of Action of *Saccharomyces cerevisiae*

In feed contaminated with AFB1, the use of *S. cerevisiae* is a method to reduce the adverse effects of aflatoxicosis (Pizzolito *et al*., 2013). Due to its desirable physiological properties, *S. cerevisiae* is the desired organism for many industrial applications and is a long history of safe use and consumption and a lack of production of toxins (Johnson and Echavarri, 2010).

S. cerevisiae cell wall is mainly made up of 80-90% polysaccharides and its mechanical resistance is due to an inside layer composed of β–Dglucans, which are formed by a complex network of highly polymerized β-(1,3)-D-glucans, branched off as β-(1,6)-D-glucans, that have a low level of polymerization (Jouany *et al.*, 2005). *S. cerevisiae* strains have been reported to bind aflatoxin B1 (AFB1) and it is an alternative method to reduce the adverse effects of aflatoxicosis, thus apart from its excellent nutritional value, yeast can also be used as a mycotoxin adsorbent (Pizzolito *et al*., 2013). *S. cerevisiae* is the efficient microorganism for removing AFB1 because it has higher values of total binding sites per cell and equilibrium constant compared to other microorganisms. The addition of *S. cerevisiae* to food would help prevent acute aflatoxicosis, chronic aflatoxicosis, or both (Bueno *et al*., 2007).

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CHAPTER THREE

THE EFFECT PERIODS OF FERMENTATION WITH AND WITHOUT *Saccharomyces cerevisiae* ON THE LEVELS OF AFLATOXIN IN MAIZE

3.1 Abstract

Aflatoxins occur as natural contaminants in cereals. Fermentation has been suggested to reduce aflatoxins in contaminated cereals. This study evaluated the effect of fermentation on the detoxification of aflatoxin-contaminated maize flour using *Saccharomyces cerevisiae*. Clean maize was moistened and inoculated with *Aspergillus flavus* then incubated at 30°C for 60 days. Three experiments were carried out, where maize flour was fermented either with or without *S. cerevisiae* for 5 and 8 days respectively, in the first and second experiments. Each time period in each treatment was replicated thrice. The ratio of water to flour in fermentation was 1.5 times that of the flour, and the aflatoxin content in the contaminated flour was 20.4 and 14.3ppb in experiments 1 and 2, respectively. In the third experiment, the flour to water ratios were 1:1 and 2:3, the fermentation period was 72 hours, and the aflatoxin content in flour was 14.8ppb. During fermentation, the pH reduced from 6.9-5.0, and the total aflatoxins reduced by 52 and 53.4% within 72 hours when fermented naturally and with *S. cerevisiae*, respectively. However, the level of aflatoxins increased after 72 hours of fermentation in experiments one and two. These results showed that natural fermentation was as effective as fermentation with *S. cerevisiae* since there were no significant differences (p>0.05) in aflatoxin concentration. It was concluded that the best ratio of flour to water for fermentation was 2:3. Furthermore, natural fermentation reduced aflatoxins by 52% after 72 hours of fermentation, while fermentation with *S. cerevisiae* did not offer any advantage since the level of reduction by 53.4% was statistically similar to 52%.

3.2 Introduction

Aflatoxins (AFs) are mycotoxins produced by some species of filamentous fungi like *Aspergillus flavus* and *Aspergillus parasiticus* (Sugiharto 2019). Temperatures ranging from 26.7 to 37.8°C and 18% moisture are optimum for *A. flavus* to grow and produce aflatoxin (Duncan and Hagler 2008) and in corn, moisture levels below 12 to 13% inhibit the growth of the fungi at any temperature (Duncan and Hagler 2008). Aflatoxins are the known class of mycotoxins in terms of toxicity and carcinogenic properties (Munkvold *et al.*, 2019). The four significant aflatoxins commonly isolated from foods and feeds are B1, B2, G1, and G2 (Obonyo and Salano 2018). Aflatoxin B1 is considered as carcinogenic mycotoxin (Kew

2013; Adelekan and Nnamah 2019) and is categorised as class1 carcinogen by the World Health Organisation (Martinez *et al.,* 2011).

Aflatoxins occur as natural contaminants of certain agricultural commodities, mainly maize, which is considered as one of the substrates for fungal growth and production of toxins (Jardon-Xicotencatl *et al.,* 2015; Chauhan *et al.,* 2016). In tropical and subtropical countries, post-harvest losses due to aflatoxins tend to be high due to lack of storage technology, equipment, and methods of reducing contamination of grain (Obonyo and Salano 2018). Additionally, there is the competition of maize for human consumption and animal feed production, which leads to a tendency for poultry feeds to be formulated using mouldy and substandard grains usually contaminated with mycotoxins (Thuita *et al.,* 2019). Therefore, there is growing concern that this contamination may be passed on to animal products in addition to reduced livestock performance (Thuita *et al.,* 2019). The United States Food and Drug Administration and European Commission recommended 20ppb total aflatoxins as the permissible level in poultry feed (FAO 2004). However, the levels of aflatoxin contamination in food (Obonyo and Salano 2018) and feed (Thuita *et al.,* 2019) usually surpass the USFDA and European Commission regulatory limits.

Fermentation has been suggested as a means of reducing occurring aflatoxin contamination in cereals (Wacoo *et al.,* 2019), and it is an inexpensive method for improving the nutritional value of feed ingredients for broiler chickens (Sugiharto and Ranjitkar 2018). The fermentation process has been employed to produce functional feed ingredients like lactic acid bacteria (LAB), lactic acid, and other organic acids that have the potential to improve gastrointestinal tract, health, immune responses, and production performance in broiler chicken (Sugiharto and Ranjitkar 2018). Yeasts have high potential applications in reducing the damage caused by toxigenic fungi, and they can degrade toxins to less-toxic or even non-toxic substances (Pfliegler *et al.,* 2015). *Saccharomyces cerevisiae* is recognised the world over for its ability to ferment sugars to ethanol and carbon dioxide (Duina *et al.,* 2014). *Saccharomyces cerevisiae* cell wall is composed of polysaccharides (80–90%) and that their mechanical strength is due to an inner layer formed by chains of β-D-glucans that bind toxins (Bovo *et al.,* 2015). Some strains of *S. cerevisiae* have been found to have high AFB1 binding capacity, which could be useful for the selection of starter cultures to prevent high aflatoxin contamination levels (Rahaie *et al.,* 2010; Johnston *et al.,* 2012). De Oliveira *et al.* (2018) suggested that the decontamination program based on the biological methods is the

best as the physical and chemical decontamination procedures may be costly and result in nutrient loss. Therefore, the objective of this study was to determine the effect and duration of fermentation using *Saccharomyces cerevisiae* on the reduction of the level of aflatoxins in contaminated maize.

3.3 Materials and Methods

3.3.1 Inoculation of maize with fungi

Clean (tested, aflatoxin-free using ELISA Test kit) maize kernels were moistened using distilled water and inoculated with a laboratory strain of *Aspergillus flavus* isolated from contaminated maize samples. The moist maize was incubated at 31°C for 60 days with periodic moistening to enable uninhibited growth of fungi and aflatoxin production. The level of AFs in the mouldy maize was determined using the ELISA technique and confirmed using liquid chromatography/Mass spectrometry (LC/MS) procedure following manufacturer's instructions (Sun *et al.,* 2015).

3.3.2 Sample extraction procedure

The maize flour samples that had been fermented and dried were used in the experiment. The ELISA (Enzyme-Linked Immunosorbent Assay Test Kit) was used following the Manufacturer's instructions (HELICA-Biosystem). The aflatoxins were extracted in 70% methanol. A 20g portion from each sample was used for analysis. The extraction solvent of 100ml was added to the 20g milled portion of the sample at the ratio of 1:5 of sample to extraction solvent (w/v). It was mixed by shaking in a vortex for 2 minutes. The particulate matter was allowed to settle, then filtered through a filter paper and the filtrate collected for determination of total aflatoxin concentrations.

3.3.3 Total aflatoxin assay procedure

All the reagents were at room temperature before use. Each standard plus the sample was placed in one dilution well placed in a microwell holder. An equal number of antibody-coated microtiter wells were placed in another microwell holder. Two hundred microlitres (200μl) of the conjugate was dispensed into each dilution well, 100μl of each standard and sample was added to appropriate dilution well containing conjugate. It was mixed by priming pipette at least three times. A new pipette tip for each was used to transfer 100μl of contents from each dilution well to a corresponding antibody-coated microtiter well and incubated at room temperature for 15 minutes. The contents from the microwells were decanted into a discard basin and washed with distilled water.

The microwells were tapped on a layer of the adsorbent towel to remove residual water. The required volume of substrate reagent (1ml/strip) was measured and placed in a separate container. One hundred microlitres (100μl) was added to each microwell and incubated at room temperature for 5 minutes, and then 100ml of stop solution was added in the same sequence as the substrate. The Absorbance optical density (OD) of each microwell was read with a Thermo Scientific™ microtiter plate reader at 450 nm. Graph pad prism7 software was used to convert the optical density (OD) data to µg/kg. The samples from ELISA were subjected to Liquid Chromatography/ Mass Spectrometry (LC/MS) analysis.

3.3.4 Fermentation

After inoculation, the contaminated maize was dried in an oven at 55^{o}C then milled into flour from which 250g was obtained and placed into a conical flask. Distilled water was added to submerge the maize sample in an airtight conical flask.

This study was carried out in three *in vitro* experiments.

Experiment one

The initial level of aflatoxins in the maize flour was 20.4ppb, and the powder to water ratio was 1:1.5 (w/v). Fermentation was carried out in two ways; through the action of native microflora (natural fermentation) and activity of yeast powder *Saccharomyces cerevisiae* National Collection of Yeast Cultures (NCYC 125®) from the Agro-Chemical & Food Company Ltd (ACFC), Kenya. The fermentation was carried out under room temperature to mimic conditions at an ordinary farm setting while following recommendations of the ACFC. During the fermentation period, pH was determined using a digital pH meter (6.7- 4.6), while the aflatoxin concentrations were measured using the ELISA technique and confirmed using the LC/MS procedure. These measurements were taken from the start of the experiment and at 24, 48, 72, 96, and 120 hours intervals. Each type (natural vs with *Saccharomyces cerevisiae*) and the period of fermentation were replicated three times. The samples were dried overnight at 55^{o}C before the total AFs were determined.

Experiment two

The initial level of AFs in the maize flour was 14.3ppb, and the flour to water ratio was 1:1.5 (w/v). Fermentation was either natural or with yeast but at an inclusion rate of 5% *S. cerevisiae* as opposed to 3% in the other two experiments. The fermentation intervals were

24, 48, 72, 96, 120, 144, 168, and 192 hours. Other fermentation conditions were similar to experiment one.

Experiment three

In the third experiment, the contaminated maize was milled into flour and fermented naturally and with yeast. The level of total AFs in the maize flour was 14.8ppb.

The ratio of maize flour to water was varied as follows:

Treatment 1 = 1:1 -1g sample: 1ml tap water - no *S. cerevisiae*

Treatment 2= 1:1 -1g sample: 1ml tap water - 3% *S. cerevisiae*

Treatment 3= 2:3 -2g sample: 3ml tap water - no *S. cerevisiae*

Treatment 4= 2:3 -2g sample: 3ml tap water - 3% *S. cerevisiae*

Each treatment was replicated three times.

3.3.5 Statistical analysis

The data were subjected to the analysis of variance using the GLM procedures of SAS, 2002 (version 9.00), and the means were separated using the paired T-Test.

The model was;

$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$

where:

Y_{ijk} = observation associated with replication k of the factor combination ij

μ = overall mean

α_i = effect of i^{th} fermentation type (i=1,2)

β_j = effect of j^{th} duration of fermentation (j=1,...,8)

$(\alpha\beta)_{ij}$ = interaction of i^{th} fermentation type with j^{th} duration of fermentation

ε_{ijk} = random error associated with Y_{ijk}

3.4 Results

The results indicate change in levels of aflatoxins during fermentation.

3.4.1 Effect of fermentation on total aflatoxins

Experiment One

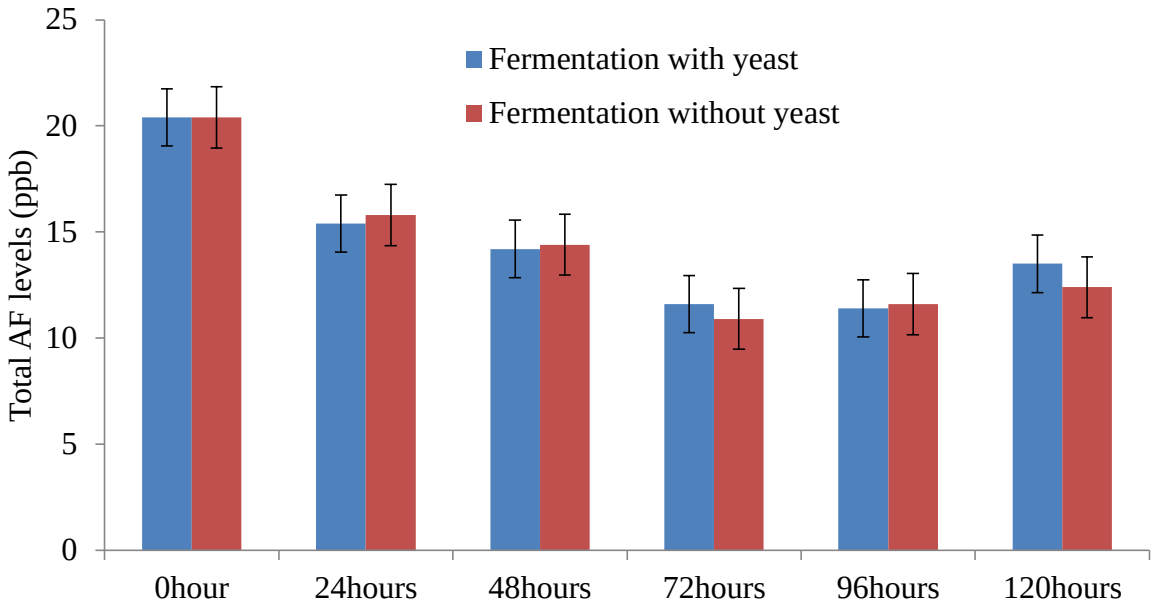

Figure 3.1 The effect of fermentation time with or without 3% Saccharomyces cerevisiae (NCYC 125®) on total aflatoxin in maize flour

The results show that the total aflatoxins in maize flour reduced over time of fermentation. The most significant reduction from 20.4ppb to 10.9ppb in aflatoxin occurred after 72 hours of fermentation Figure 3.1. However, there was no significant difference ($p>0.05$) between natural and fermentation with 3% *Saccharomyces cerevisiae.* After 72 hours, the total aflatoxin concentration increased.

Experiment two

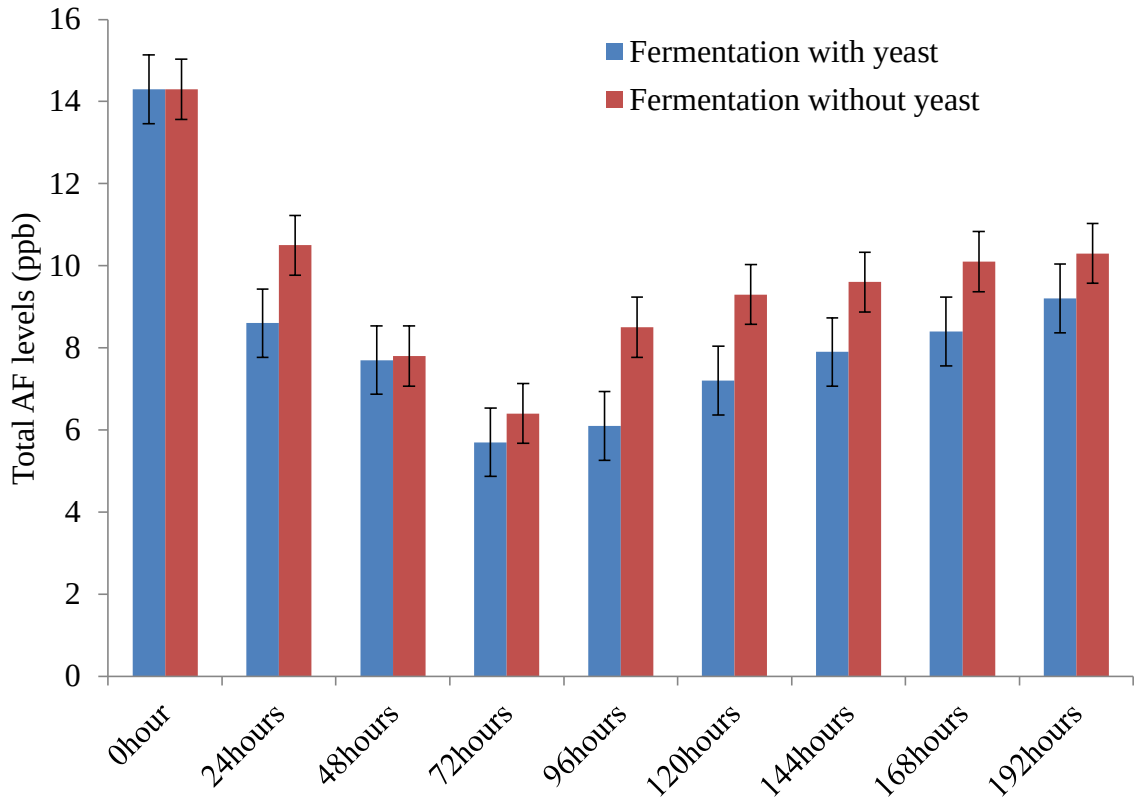

Figure 3.2 *The effect of fermentation of maize flour with or without 5% Saccharomyces cerevisiae* (NCYC 125®) *on the levels of total aflatoxin*.

After 72 hours of fermentation, the total aflatoxins decreased by 60.1 and 55.2% with and without *Saccharomyces cerevisiae*, respectively, and then increased when the fermentation period continued. When maize was fermented with and without yeast, the total AF levels were different ($p<0.05$) after 24, 96, 120, 144, and 168 hours but similar after 48, 72, and 192 hours of fermentation. After 72 hours and 192 hours of fermentation, the total AF levels were identical. After 72 hours, the aflatoxin content is decreased irrespective of fermentation type (with or without 5% *Saccharomyces cerevisiae*) (Figure 3.2).

Experiment three

The results indicate that the substrate to water ratio of 2:3 is better than 1:1 in reducing the amount of aflatoxin (Table 3.1).

Table 3.1 Reduction of total aflatoxins after 72 hours of fermentation using different substrate: water ratios

	Fermentation type	
Flour: water ratio	With 3% *S. cerevisiae*	Without *S. cerevisiae*
1:1	41.4±10.1	32.8±7.7
2:3	53.7±9.9	51.3±8.4

±: standard deviation

3.4.2 Effect of fermentation on pH

In the three experiments, the pH of fermented mixtures decreased from 6.7 to 5 and 5.2 in 72 hours of natural fermentation or with *S. cerevisiae* (Figure 3.3).

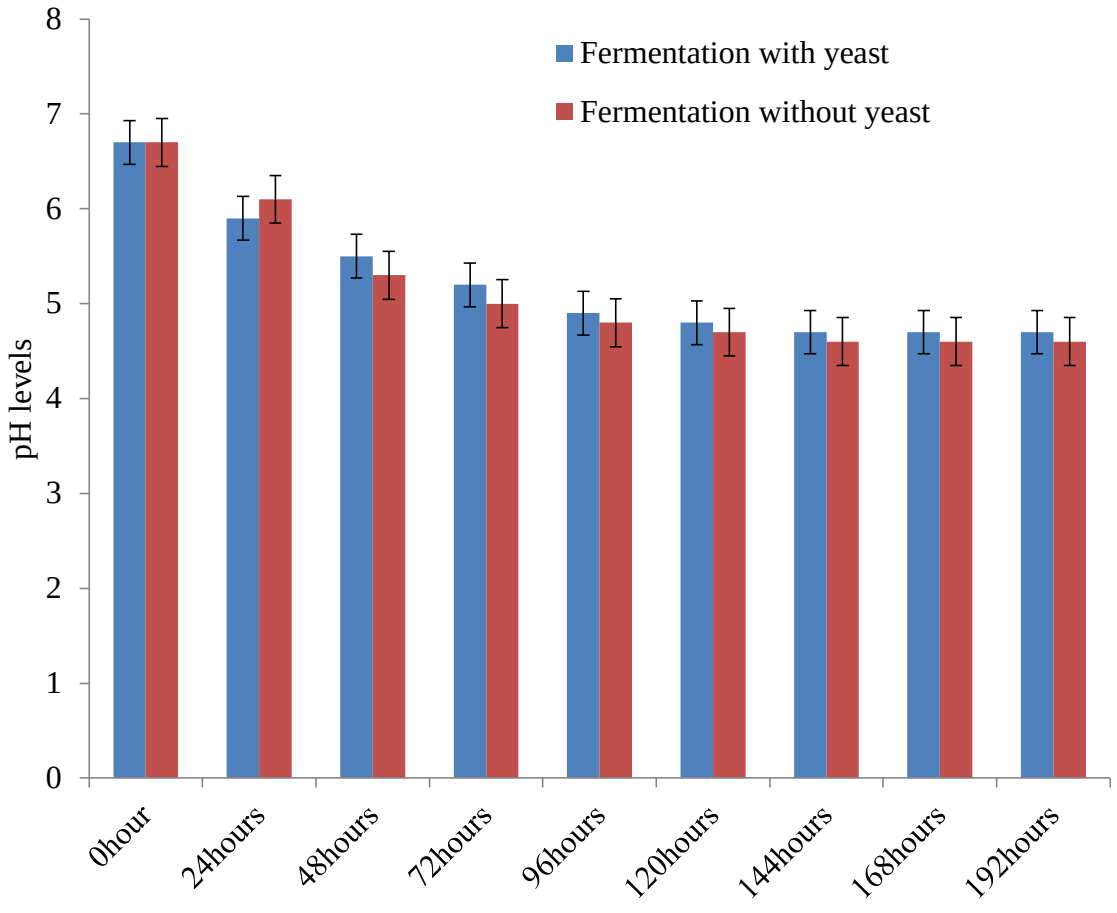

Figure 3.3 Levels of pH during 8days of fermentation

The pH was almost constant after 72 hours of fermentation. The decrease in pH was similar irrespective of the type of fermentation (natural or with *S. cerevisiae*).

Table 3.2 Reduction of total aflatoxins after 72 hours of fermentation in the three different experiments

Experiment No	Flour: water ratio	Fermentation periods	Fermentation Types		
			With *S. cerevisiae*		Without *S. cerevisiae*
			% of yeast		
Experiment 1	2:3	120 hours	3%	46.5±2.4	49.5±0.7
Experiment 2	2:3	192 hours	5%	60.1±6.0	55.2±3.7
Experiment 3	1:1 & 2:3	72 hours	3%	53.7±9.9	51.3±8.4
Average				53.4±6.1	52.0±4.2

±: standard deviation

In the first and third experiments, fermentation using the 3% *S. cerevisiae* was as effective as fermentation without yeast (natural fermentation). Fermentation using 5% *S. cerevisiae* in the second experiment reduced total aflatoxins by 60.1% compared to natural fermentation, where the reduction was 55.2%. Overall, the average reduction in total aflatoxins in the three experiments was 53.4 and 52% using *S. cerevisiae* and natural fermentation, respectively. The decrease was not significantly different (p>0.05).

3.5 Discussion

Mitigation measures against aflatoxins (AF) are desirable considering their adverse effects in poultry production like compromising of important production parameters including weight gain, feed intake, feed conversion efficiency, and reproductive performance (Suhaimi *et al*., 2017). Several strategies have been developed and tested to reduce AF levels in animal feed, but their adoption is low due to either high costs or technical difficulties (Udomkun *et al***.,** 2017). In this study, the effect of fermentation on AF levels in contaminated maize was evaluated. The method chosen was to mimic what is easily implemented among subsistence farmers. This is because it is more practical to ferment contaminated ingredients rather than the complete poultry feed. The use of yeast in the fermentation of animal feed ingredients is a possible means of reducing widely occurring aflatoxin contamination (Hayo, 2018; Wacoo *et al***.,** 2019).

The findings of this study demonstrate that spontaneous fermentation through the action of indigenous microflora and fermentation using *S. cerevisiae* reduced levels of total aflatoxins by 52.1 and 53.5% respectively (Table 3.2). The levels of aflatoxin in maize flour before fermentation was considered unsafe (20.4 and 14.3ppb), but these reduced to safe (10.9 and 5.7ppb) (Figure 3.1 & 3.2) levels for poultry feed formulation (FAO, 2004). The

highest reduction of total aflatoxin occurred within 72 hours of fermentation which corroborates other studies. Adeleken and Nnamah (2019) reported a reduction in the aflatoxin content of maize from an initial concentration of 58.00 to 3.1ppb in a similar steeping period. Similar findings have also been reported by Assohoun *et al.* (2013); Okeke *et al.* (2015); Poloni *et al.* (2017).

It has been suggested that *S. cerevisiae* binds toxic metabolites of filamentous fungi to the cell wall, thereby significantly reducing AFB1 concentrations (Gonçalves *et al.*, 2015; Chlebicz and Śliżewska 2019). Similarly, spontaneous natural fermentation without yeast has been shown to reduce levels of total aflatoxins. This is thought to be due to lactic acid bacteria that remove toxins through non-covalent binding of mutagens by fractions of the cell wall skeleton of the lactic acid bacteria (Zhang and Ohta, 1991). However, another alternative mechanism of aflatoxin B1 removal has been reported in which lactic acid bacteria fermentation opens up the aflatoxin B1 lactone ring resulting in its complete detoxification (Nout 1994). This study design was not to explain the mode of action of removal of aflatoxins neither account for the observed difference in aflatoxin reduction in the two methods chosen.

The findings of this study demonstrate that during fermentation using both methods, the ratio of flour to water (1:1 and 2:3) affected ($p<0.05$) the final aflatoxin levels (Table 3.1). The highest reduction in total aflatoxin levels was observed in fermentation with the ratio of 2:3 (2g sample: 3ml tap water) (Table 3.1). This corroborates the work of Biernasiak *et al.*, (2006) who used the same ratio to detoxify mycotoxins using probiotic preparation for broiler chickens. There was a drop in pH after fermentation as compared to the pH of non-fermented samples (Figure 3.3). This was due to metabolic processes releasing organic acids like acetic acid and ethanol and a few other minor products (Műller 2008). Due to detectable amounts of lactic and acetic acids after fermentation, lactic acid bacteria dominate the culture system and result in lower pH, an essential characteristic for product safety (Poloni *et al.*, 2017). The low pH of fermented feeds acidifies the upper digestive tract and thereby improves the barrier function of the gizzard against pathogens (Sugiharto and Ranjitkar 2018). Additionally, it has been reported that the low pH in fermented feeds increases the resistance of poultry diets to fungal contamination (Londero *et al.*, 2014).

3.6 Conclusions

The best period of fermentation to reduce the total aflatoxin levels in maize was achieved after 72 hours in 2:3 substrate to water ratio.

3.7 Recommendation

There is a need for further research to investigate the best fermentation period (hours) of contaminated maize under different fermentation conditions and yeast strains.

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CHAPTER FOUR

THE EFFECT OF FERMENTATION USING *Saccharomyces cerevisiae* ON DRY MATTER DIGESTIBILITY, METABOLIZABLE ENERGY AND NITROGEN IN BROILER CHICKENS

4.1 Abstract

Poultry is highly susceptible to mycotoxicoses caused by aflatoxins. Aflatoxin producing fungi utilise the nutrients present in the ingredients for their metabolism and propagation, and thereby reduce the nutritional quality of the feed. In a previous experiment, fermentation was found to decrease the level of aflatoxins in contaminated feed ingredients. Fermented feeds have also been reported to improve the performance and intestinal health of broilers. The objective of this study was to evaluate the effect of fermented feed on the digestibility of dry matter, metabolizable energy content, and nitrogen utilisation in broiler chickens. Twenty-four, 28-day old male broiler chickens were assigned to six different dietary treatments. Each dietary treatment was assigned to four chickens and fed the diets for 14 days (7 days adaptation period and 7 days total faecal collection period). The faeces were oven-dried daily after collection and pooled per cage, for the determination of dry matter, metabolizable energy and nitrogen digestibility. The dietary treatments were: - Diet 1 (No aflatoxin and not fermented), diet 2 (No aflatoxin and fermented without *S. cerevisiae*), diet 3 (No aflatoxin and fermented with *S. cerevisiae*), diet 4 (Contained 20.034ppb aflatoxin and not fermented), diet 5 (Contained 20.034ppb aflatoxin and fermented without *S. cerevisiae*) and diet 6 (Contained 20.034ppb aflatoxin and fermented with *S. cerevisiae*). The results showed that dry matter digestibility and metabolizable nitrogen were significantly (p<0.05) affected by fermentation type. The dry matter digestibility was decreased by fermentation with *S. cerevisiae* decreased (63.7%) but increased by fermentation without *S. cerevisiae* the metabolizable nitrogen (68.7%) of the diets. The dry matter digestibility of the diet was not affected by total aflatoxin levels, but metabolizable energy and nitrogen digestibility were significantly (p<0.05) affected by the overall aflatoxin levels in the diet. Fermentation with and without *S. cerevisiae* had a similar (p>0.05) effect on metabolizable energy and nitrogen. It is therefore concluded that natural fermentation is the best method to improve feed digestibility. Other studies can be carried out on using the digestibility of nitrogen as a measure to find out the amount of total aflatoxins which does not cause injury of the pancreas.

4.2 Introduction

Aflatoxins were discovered in 1960 and are widely associated with the maize, groundnuts, tree nuts, figs, dates, and certain oil seeds such as cotton seeds (Kanyi, 2018; Negash, 2018). The four most common aflatoxins in the feed are B1, B2, G1, and G2, which interfere with the metabolism of carbohydrates, fats, and nucleic acids in livestock (Negash, 2018). Growth of toxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* on maize often results in the production of aflatoxin B1, the most biologically active member of the aflatoxin family (Zaki *et al.,* 2012). Maize is the most commonly used feed ingredient in poultry diets and is more susceptible to fungi contamination and hence, aflatoxin production throughout the world (Fareed *et al*., 2014). Chickens are highly vulnerable to mycotoxicoses caused by aflatoxins (Anjum *et al.,* 2012). According to many regulatory bodies on aflatoxins, tolerable levels of total aflatoxins in foodstuffs and animal feeds are different. The World Health Organization (WHO) set aflatoxins limits of 5ppbfor animals (Kajuna *et al*., 2013) while United States Food and Drug Administration and European Commission have set 20ppb as the worldwide range of maximum tolerable level of total aflatoxins for poultry feed (Syahidah *et al.,* 2017).

The presence of aflatoxins in feed ingredients affects poultry because of their higher susceptibility to aflatoxins (Anjum *et al.*, 2012). Aflatoxin B1 (AFB1) in the edible parts of a bird is recognised as a carcinogenic substrate as it reacts with DNA to induce mutations, which can lead to cancer. In general, the concentrations of AFB1 in poultry meat and the edible parts may be elevated irrespective of whether the aflatoxin levels in the diet is low (22 ppb) or high (2500 ppb) (Fouad *et al.,* 2019). Furthermore, mycotoxins have adverse effects on intestinal health by decreasing cell viability, reductions in short-chain fatty acid concentrations, and the elimination of beneficial bacteria (Broom, 2015).

Fermentation is a metabolic process by which organic molecules, typically glucose, are converted into acids, gases, or alcohol in the absence of oxygen and may be used to improve the performance and intestinal health of broilers (Kim and Kang, 2016). Animal feed manufacturing industries compete for feed materials with humans. Thus, low-quality raw materials are often used for poultry feed production (Abidin *et al.,* 2011). Fermentation is a viable method of reducing levels of non-nutritive compounds and improves the overall nutritive value of the feed (Aljuobori *et al.,* 2014; Çabuk *et al*., 2018). Aflatoxin producing fungi utilise the nutrients in the ingredients for their metabolism and propagation, and thereby

reduce the nutritional quality of the feed ingredients (Akande *et al.,* 2006). Sugiharto *et al.* (2015) suggested fermenting the grain fraction only (before incorporation into compound diets) instead of the complete foods to avoid losses of some essential nutrients in fermented feeds. The use of fermented liquid feed appears to be a cost-effective alternative to the use of antibiotics growth promoters (Missotten *et al*., 2015). Fermentation reduces AF level as in experiment one in chapter three above. The aim of the current investigation was to evaluate the effect of fermentation on the digestibility of dry matter, metabolizable energy, and nitrogen in broiler chicken.

4.3 Materials and Methods

4.3.1 Study site

The experiment was conducted at the Poultry Unit, Tatton Agricultural Park of Egerton University, Nakuru County. Egerton is located at longitude 35°57'E and latitude 0°23'S with an altitude of 2,238m above sea level. It has a mean daily temperature of 21°C. There is a bimodal rainfall pattern (March to May and June to September) with a mean annual rainfall of 900 - 1,020mm (Egerton University, Meteorological Station, 2018). The study was undertaken from 12th July to 26th July 2019. The room temperature during the experimental period was 24-26°C.

4.3.2 Source of aflatoxin

Clean (tested, aflatoxin-free) maize kernels were inoculated with *Aspergillus flavus.* After incubation, the level of total AFs was determined using the ELISA technique following the manufacturer's instructions. The total aflatoxin level in maize used to formulate contaminated feed was 28ppb. After the formulation of feed for broiler chicks, the level of aflatoxins in contaminated feeds was 20.034ppb.

4.3.3 Fermentation

Both the contaminated and clean maize was milled using a sieve of 0.8mm into flour, tap water was added at the ratio of 1:1.5 (w/v) weight of maize flour: volume of water. The fermentation of contaminated and clean maize flour was done with and without *Saccharomyces cerevisiae* (NCYC 125®) at ambient room temperature for 72 hours, and then solar dried. After drying, the fermented maize flour was used to compound the six different broiler diets.

4.3.4 Experimental diets, animals, design and treatments

The diets were formulated to meet the NRC (1994) requirements for finisher broiler chickens of 180g/kg crude protein and about 3200Kcal/kg metabolizable energy (Table 4.1). The diets were:

Diet 1 - No aflatoxin and not fermented,
Diet 2 - No aflatoxin and fermented without *S. cerevisiae*,
Diet 3 - No aflatoxin and fermented with *S. cerevisiae*,
Diet 4 - Contained 20.034ppb aflatoxin and not fermented,
Diet 5 - Contained 20.034ppb aflatoxin and fermented without *S. cerevisiae*,
Diet 6 - Contained 20.034ppb aflatoxin and fermented with *S. cerevisiae*.

Twenty four, 28-day old male broiler chickens with similar body weights (950±15g) were obtained from a farmer within Nakuru County. The chickens were housed in a well-ventilated room that had been cleaned with liquid soap and disinfected before the introduction of the chickens. Chickens were randomly assigned to six dietary treatments in a Completely Randomized Design (CRD) where each diet was assigned four chickens. The chickens were allowed seven days adaptation period and seven days total faecal collection period.

Table 4.1 Composition of experimental diets for broiler chicken

Ingredients(g/100g)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Maize	71.55	71.55	71.55	71.55	71.55	71.55
Vegetable oil	2	2	2	2	2	2
Soya bean meal	22	22	22	22	22	22
Omena[1]	2	2	2	2	2	2
Dicalcium Phosphate	1.2	1.2	1.2	1.2	1.2	1.2
Limestone	0.45	0.45	0.45	0.45	0.45	0.45
Iodized salt	0.3	0.3	0.3	0.3	0.3	0.3
Mineral and Vitamin Premix[2]	0.5	0.5	0.5	0.5	0.5	0.5
Totals	100	100	100	100	100	100
Calculated ME(Kcal/kg)	3174.8	3174.8	3174.8	3174.8	3174.8	3174.8
Calculated CP (g/kg)	179	179	179	179	179	179
Total AFs content in the diet (ppb)	0	0	0	20.034	20.034	20.034
Fermentation types	a	b	c	a	b	b

[1]*Scientific name: Rastrineobola Argentea, common name; silver cyprinid, and it's also called the Lake Victoria sardine or mukene.*

[2]*Supplied the following per Kg of diet: Vit. A = 10,000 IU; Vit. D = 2,800 IU; Vit. E = 24mg; Vit. K = 2mg; Vit. B1 = 1mg; Vit. B2 = 4mg; Vit. B6 = 4mg; Vit. B12 =16mg, Vit. C = 200mg; Niacin = 10mg; Folic acid =1.6mg; Biotin= 60mg; Mn = 80mg; Fe = 25mg; Cu = 5mg; Zn = 50mg; Se=200mg and KI=1.2mg.*

a = not fermented, b = fermented without S. cerevisiae, c = fermented without S. cerevisiae.

Diets were offered to the chicken *ad libitum* daily at 09:00h using clean disinfected feeders. Any leftover feed was collected before the next feeding. Clean drinking water was provided *ad libitum* in water drinkers throughout the experiment.

4.3.5 Data collection and sample analysis

Faeces and leftover feed from each cage were collected daily before feeding at 9.00h. The faeces were dried at 55^{O}C for 48h, ground through a 0.5mm screen before analysis. The nitrogen in the feed and faeces was analysed by the Kjeldahl method using the (AOAC, 2006). Gross energy was determined using a bomb calorimeter "e2K series" (www.cal2k.com, South Africa). The apparent dry matter digestibility (DMD), metabolizable energy (ME) and nitrogen (MN) were calculated as follows:

$$Dry\ matter\ digestibility = \frac{\text{Dry matter intake} - \text{Dry matter faeces}}{\text{Dry matter intake}}$$

$$Metabolizable\ energy\ = \frac{\text{Gross energy in the diet} - \text{Gross energy in the faeces}}{\text{Gross energy in feed consumed}}$$

$$Metabolizable\ nitrogen = \frac{\text{Nitrogen in the diet} - \text{Nitrogen in the faeces}}{\text{Nitrogen in the diet}}$$

4.3.6 Statistical analysis

Data were subjected to 1- way analysis of variance (ANOVA) using the General linear model (GLM) of Statistical Analysis Systems (SAS, 2009) software. An F-test at a 5% probability level was used to test for significance and means separation was done by Least Significance Difference Procedure.

4.3.7 Statistical model

$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$

where:

Y_{ij} = observation on j^{th} chicken of i^{th}dietary treatment

μ = overall mean

τ_i = effect due to i^{th} dietary treatment, (i=1,2,3,4,5,6)

ε_{ij} = random error associated with Y_{ij}

4.4 Results

The results of this experiment are summarised in Tables 4.2, 4.3, 4.4, and 4.5.

Table 4.2 The effect of dietary treatment on dry matter digestibility in broiler chickens (n=4)

Diet	Fermentation treatment	Total aflatoxin level (ppb)	DMD means± Standard deviations
Diet 1	Not fermented	0	65.1±3.74
Diet 2	Fermented without *S. cerevisiae*	0	69.1±2.37
Diet 3	Fermented with *S. cerevisiae*	0	63.9±2.51
Diet 4	Not fermented	20.034	69.9±2.64
Diet 5	Fermented without *S. cerevisiae*	20.034	68.2±5.56
Diet 6	Fermented with *S. cerevisiae*	20.034	63.5±1.89

The dry matter digestibility was decreased in contaminated and non-contaminated diet fermented with *S. cerevisiae*.

Table 4.3 The effect of dietary treatment on the metabolizable energy (ME) value in broilers

Diet	Fermentation treatment	Total Aflatoxin level (ppb)	ME means± Standard deviations (%)
Diet 1	Not fermented	0	20.5±2.28
Diet 2	Fermented without *S. cerevisiae*	0	26.2±10.73
Diet 3	Fermented with *S. cerevisiae*	0	25.5±0.26
Diet 4	Not fermented	20.034	12.1±1.58
Diet 5	Fermented without *S. cerevisiae*	20.034	11.9±2.74
Diet 6	Fermented with *S. cerevisiae*	20.034	13.9±2.22

The total aflatoxin levels affected the metabolizable energy of the diet, irrespective of whether the diet was fermented naturally or with *Saccharomyces cerevisiae* (NCYC 125®).

Table 4.4 The effect of dietary treatment on the metabolizable nitrogen (MN) value in broilers

Diets	Fermentation treatment	Total AFs level (ppb)	MN means ± Standard deviation (%)
Diet 1	Not fermented	0	27.6±7.14
Diet 2	Fermented without *S. cerevisiae*	0	38.7±3.50
Diet 3	Fermented with *S. cerevisiae*	0	43.1±4.19
Diet 4	Not fermented	20.034	48.2±6.49
Diet 5	Fermented without *S. cerevisiae*	20.034	48.3±10.05
Diet 6	Fermented with *S. cerevisiae*	20.034	50.3±5.03

The fermentation with and without *Saccharomyces cerevisiae* (NCYC 125®) improved the metabolisable nitrogen of the diet, irrespective of whether the diet was contaminated with 20.034ppb of total aflatoxins or not.

Table 4.5 The effect of fermentation types and total AFs levels on the digestibility of dry matter (DMD), metabolizable energy (ME) and nitrogen (MN)

Fermentation types				
Nutrients	Not fermented	Fermented without *S. cerevisiae*	Fermented with *S. cerevisiae*	*p*-values
DMD	67.5[a]	68.7[a]	63.7[b]	0.0023
ME	16.3[a]	19.1[a]	19.7[a]	0.3208
MN	37.9[b]	43.5[a]	46.7[a]	0.0128
Total aflatoxin levels				
Nutrients	0ppb	20.034ppb	*p*-values	
DMD	66.0[a]	67.2[a]	0.4032	
ME	24.1[a]	12.7[b]	<.0001	
MN	36.5[a]	48.9[b]	<.0001	

[a, b] means in the same row with different superscripts are significantly different ($p < 0.05$)

The dry matter digestibility and metabolizable nitrogen were affected considerably by fermentation types (Table 4.5). Natural fermentation improved dry matter digestibility of the diet with or without. Both fermentation offered the same advantages on metabolizable energy

and nitrogen. The dry matter digestibility was not affected by total aflatoxin levels. Both metabolizable energy and nitrogen were significantly affected by the total aflatoxin levels in the diet.

4.5 Discussion

The dry matter digestibility (DMD) was affected by the fermentation type (Table 4.2). The natural fermentation improved the DMD in the non-contaminated and contaminated diet (20.034ppb total aflatoxin) compared to fermentation with *S. cerevisiae*. The DMD was 69.1 and 68.2% in a non-contaminated and contaminated diet fermented naturally, respectively, while in fermentation with *S. cerevisiae*, the DMD in the non-contaminated and contaminated diet was 63.9 and 63.5% respectively. The DMD was not affected by the total aflatoxin levels in the menu. These results are in agreement with the findings of Applegate *et al.* (2009), who reported no effect of 600, 1200, and 2500ppb AFB1 levels in diet on the digestibility of dry matter.

The fermentation types (fermentation with and without *S. cerevisiae*) significantly affected the dry matter digestibility (p=0.0023), where natural fermentation showed higher dry matter digestibility compared to fermentation with *S. cerevisiae* (Table 4.5). This observation corroborates that of Lee *et al.* (2017), who reported that the dietary inclusion of fermented products significantly improved hemicellulose digestibility, allowing broilers on a supplemented diet to maintain a duodenal and jejunal weight. The dry matter digestibility in the non-fermented contaminated diet was higher in this study. This was also reported by Abbasi *et al.* (2018), who found an improved apparent ileal dry matter digestibility. This was as a result of increased digestive enzymes activity due to aflatoxins exposure as reported by other studies. Han *et al.* (2008) indicated that increased digestive enzymes were released from the injured pancreas in response to AFB_1. They suggested that increased pro-enzymes released from the injured pancreas during aflatoxicosis were a plausible reason. A significant effect of AFB1 on pancreatic amylase and lipase release was observed with birds fed aflatoxin-contaminated diets showing higher enzyme activity during aflatoxicosis (Chen *et al*., 2016).

Metabolizable energy (ME) of contaminated feed was not affected whether feed was naturally fermented or fermented with *S. cerevisiae* (Table 4.3). Aljuobori *et al.* (2014) found that apparent metabolizable energy was not affected by fermentation. The total aflatoxin levels significantly (p<.0001) decreased the metabolizable energy (Table 4.5). The

observation corroborates the findings of Applegate *et al.* (2009), who reported a reduction of the apparent metabolizable energy at 600 and 1200ppb of AFB1 in the diet. This is the result of the down-regulation of various hepatic genes associated with energy production and fatty acid metabolism (Murugesan *et al.* (2015).

In this study, fermentation types improved the metabolizable nitrogen in a non-contaminated diet from 27.7% (no fermented clean diet) to 38.8 and 43.0% in a diet fermented naturally and fermented with yeast, respectively (Table 4.4). Fermentation with *S. cerevisiae* improved the metabolizable nitrogen (Table 4.5) because *Saccharomyces cerevisiae* is an excellent source of protein. It was observed that the *S. cerevisiae* used during fermentation is a rich source of protein with 50% protein of its biomass and it has also a high content of lysine (Onofre *et al.,* 2017). This proves *S. cerevisiae* to be a special protein supplement to be used in different sectors, such as the production of animal feed. This study showed a significant (p <.0001) effect of total aflatoxin levels on metabolizable nitrogen whether fermented or not (Table 4.5). This was a result of metabolites produced during fermentation. However, the metabolizable nitrogen in the non-contaminated diet was lower compared to a contaminated food with 20.034ppb. This is contrary results reported by other studies that reported a decrease of metabolizable nitrogen in the contaminated diet by aflatoxins. Fouad *et al.* (2019) reported that poultry liver, a central organ for lipid, protein, and amino acid metabolism and their utilisation is affected by diets containing AFB1, which diminish protein and lipid biogenesis of poultry fed such diets. Salem *et al.* (2018) also reported that feed contaminated with high aflatoxin B1 (250ppb) in the diet significantly decreased total serum protein. Han *et al.* (2008), reported the same where they found a low concentration of AFB1 (20ppb) produced by *A. flavus* in the diet of ducks for six weeks led to a significant increase in the relative weight of the pancreas which increased the activities of digestive enzymes (amylase, lipase, protease, chymotrypsin, and trypsin) and decreased the apparent digestibility of crude protein.

4.6 Conclusion

Fermentation with *Saccharomyces cerevisiae* (NCYC 125®) has decreased the dry matter digestibility, increased the metabolizable nitrogen content on whether there is aflatoxins or not, but it has not improved the metabolizable energy.

4.7 Recommendation

Conduct further studies on using digestibility of Nitrogen as a measure to find out the amount of total aflatoxins which does not cause injury of the pancreas

4.8 References

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CHAPTER FIVE

FEED INTAKE AND GROWTH OF BROILER CHICKEN OFFERED FERMENTED FEED WITH OR WITHOUT *Saccharomyces cerevisiae*

5.1 Abstract

Broiler meat is a source of protein with high biological value and micronutrient content, such as vitamin A, thiamine, iron, phosphorus, and nicotinic acid. Aflatoxins in broiler chicken impair production parameters which result in economic losses. The current study evaluated feed intake and growth of broiler chicken offered fermented feed with or without *Saccharomyces cerevisiae*. One hundred and forty-four (144) broiler chicks were fed on six different diets: diet 1 (no aflatoxin and not fermented), diet 2 (no aflatoxin and fermented without *S. cerevisiae*), diet 3 (no aflatoxin and fermented with *S. cerevisiae*), diet 4 (contained 30.08ppb aflatoxin and not fermented), diet 5 (contained 30.08ppb aflatoxin and fermented without *S. cerevisiae*) and diet 6 (contained 30.08ppb aflatoxin fermented with *S. cerevisiae*). Each diet was assigned to six chicks, replicated four times. In a completely randomised design, the chicks were assigned to six diets from day one to 21 days. Leftover feed was recorded daily, and chicks were weighed on a weekly basis. Mortalities were recorded when they occurred. Daily feed intake was calculated as feed offered minus leftovers. Bodyweight gain was calculated as final weight minus initial weight and gain: feed ratio as the body-weight gain divided by feed intake. There was no significant difference (p>0.05) in feed intake and body weight gain. However, gain: feed ratio was significantly (p=0.048) better in broilers fed diets which were fermented without *S. cerevisiae*. These results showed that natural fermentation was as effective as that with *S. cerevisiae*. The mortality rate was 75.0% in chicks fed on the diet containing 30.08ppb, which was not fermented. However, the mortality rate was very low in contaminated feed fermented naturally (29.2%) compared to that fermented with *S. cerevisiae* (58.3%). Therefore, fermentation without *S. cerevisiae* (natural) is a suitable method of improving the quality of aflatoxin-contaminated feed for broilers. It is recommended that another study be to carry out to determine the effect of feeding fermented feed on the quality of broiler meat.

5.2 Introduction

Throughout the world, poultry meat consumption continues to grow, both in developed and in developing countries (Kralik *et al.,* 2018). Broiler chicken farming is a significant sector of the poultry industry. Chicken meat is a source of protein with high

biological value (Da Silva *et al.,* 2017) and moderate energy value places chicken meat as healthy food (Marangoni *et al.,* 2015). Poultry meat is recommended for use in healthy diets due to its reduced fat content, as well as a higher proportion of polyunsaturated fatty acids (PUFA) when compared to other species' meats (Riovanto *et al.,* 2012). Contamination of poultry feeds during storage, by bacteria, moulds, and fungi may cause spoilage which adversely affects feed quality as well as increasing poultry risks towards infections (Sugiharto and Ranjitkar, 2018). Aflatoxin B1 toxicity in broilers causes significant economic losses and is harmful to public health (Yunus *et al.,* 2011). In poultry, total aflatoxin impairs most of the critical production parameters, including weight gain, feed intake, feed conversion efficiency and yield (Hussain *et al.,* 2010). In 2003, Kenya was one of the five African countries to report standards on aflatoxins, and the acceptable limit of total aflatoxins contamination in cereals is 10ppb (Sirma *et al.,* 2018). Kenyan standards likely match East African Standards which specify maximum aflatoxin limits as 20ppb and 10ppb AFB1 for adult and young poultry feed respectively (Sirma *et al.,* 2018).

Fermentation is the chemical transformation of organic substances into simpler compounds by the active enzymes, complex biological catalysts, produced by microorganisms such as bacteria, yeasts, or moulds (Jawad *et al*., 2016). Fermented feed influenced the bacterial ecology of the gastrointestinal tract and reduces the level of Enterobacteriaceae in different parts of the gastrointestinal tract of broiler chicks (Heres *et al.,* 2003). The fermented feed causes a reduction of pathogenic bacteria, including Salmonella and Campylobacter in the digestive tract, most notably in the crop and gizzard (Jawad *et al.,* 2016). Yeast-based products were reported to have potential application in animal feeds as a suitable biological method for reducing the adverse effects of aflatoxins (Bovo *et al.,* 2015). The addition of yeast to broiler diets containing AFB1 was shown to be effective in ameliorating the toxin's adverse effects on productive parameters (average daily weight gain, average daily consumption, feed conversion ratio and carcass weight) and residual AFB1 levels in the liver (Magnoli *et al.,* 2017). This experiment aimed to determine the effect of fermentation of aflatoxin-contaminated maize with and without *Saccharomyces cerevisiae* on growth, feed intake and feed efficiency of broilers.

5.3 Materials and Methods

5.3.1 Study site

The study was conducted at the Indigenous Chicken Improvement Programme (INCIP) Poultry Unit, Tatton Agricultural Park of Egerton University, Nakuru County. Egerton is located at longitude 35º57'E and latitude 0º 23'S with an altitude of 2,238m above sea level. It has a mean daily temperature of 21^{o}C. There is a bimodal rainfall pattern (March to May and June to September) with a mean annual rainfall of 900 - 1,020mm (Egerton University, Meteorological Station, 2018).

5.3.2 Source of aflatoxin

Clean (tested, aflatoxin-free) maize kernels were inoculated with *Aspergillus flavus* isolated from contaminated maize samples. The maize was then incubated at 31^{o}C for 60 days with periodic moistening to enable uninhibited growth of fungi and aflatoxin production. The level of AFs in the maize was determined using the ELISA technique following the manufacturer's instructions. The total aflatoxin level in maize used to formulate contaminated feed was 43.6ppb. After formulation of feed for broiler chicks, the level of aflatoxins in contaminated feeds was 30.08ppb.

5.3.3 Fermentation

After inoculation, the contaminated maize and clean maize was milled using a sieve of 0.8mm into flour from which the water was added to the maize flour in the ratio of 1:1.5w/v (weight of maize flour/volume of water). The fermentation was done either without or by adding *S. cerevisiae* (NCYC 125®) at room temperature (25^{o}C) for 72hours, then sun-dried. After drying, fermented maize flour was used to compound six experimental diets.

5.3.4 Experimental animals and management

One hundred and forty-four (144), day-old broiler chicks were purchased from a commercial hatchery (Kenchic). All vaccination procedures against Gumboro and New Castle diseases were carried out by the hatchery before supply. The chicks were then put in a room which was well ventilated and fitted with fluorescent lighting. It was cleaned with liquid soap and disinfected before occupation by the chicks. The chicks were weighed individually before feeding. During brooding, the room was warmed to 30-34^{o}C using infrared bulbs, and there was continuous lighting. In the poultry house, 24 partitions with (1.2m x 1.2m each) were made.

Each diet was assigned to 6 broiler chickens of similar body weights and replicated four times. Chickens were offered the six diets for 21 days. The six experimental diets were offered in clean disinfected feeders daily at 09.00hrs. The leftover feed was collected, weighed, recorded, and fresh feed provided before the next feeding. Clean drinking water was provided *ad libitum* throughout the experimental period.

5.3.5 Dietary treatments and experimental design

The diets were formulated to meet the NRC (1994) requirements for starter broiler chicken of 230g/kg crude protein and about 3200Kcal/kg metabolizable energy. The experimental design was a "three by two factorial" arrangement where factor one was fermentation type (not fermented, fermented without *S. cerevisiae* and fermented with *S. cerevisiae*) and factor two was aflatoxin (0ppb and 30.08ppb). The six experimental diets are shown in Table 5.1.

Diet 1 - No aflatoxin and not fermented,
Diet 2 - No aflatoxin and fermented without *S. cerevisiae*,
Diet 3 - No aflatoxin and fermented with *S. cerevisiae*,
Diet 4 - Contained 30.08ppb aflatoxin and not fermented,
Diet 5 - Contained 30.08ppb aflatoxin and fermented without *S. cerevisiae*,
Diet 6 - Contained 30.08ppb aflatoxin fermented with *S. cerevisiae*.

Table 5.1 Composition of experimental diets for starter broiler chicks in g/100g

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Maize	69	69	69	69	69	69
Soy Bean Meal	6	6	6	6	6	6
Omena[1]	23	23	23	23	23	23
Dicalcium-Phosphate	1	1	1	1	1	1
Limestone	0.25	0.25	0.25	0.25	0.25	0.25
Iodized salt	0.25	0.25	0.25	0.25	0.25	0.25
Mineral and Vitamin Premix[2]	0.5	0.5	0.5	0.5	0.5	0.5
Totals	100	100	100	100	100	100
Calculated ME(Kcal/kg)	3076	3076	3076	3076	3076	3076
Calculated CP (g/kg)	226	226	226	226	226	226
Calculated AFs content in diets (ppb)	0	0	0	20.034	20.034	20.034
Fermentation types	a	b	c	a	b	c

[1]*Scientific name: Rastrineobola Argentea, common name; silver cyprinid, and it's also called the Lake Victoria sardine or mukene,*

[2]*Supplied the following per Kg of diet: Vit. A = 10,000 IU; Vit. D = 2,800 IU; Vit. E = 24mg; Vit. K = 2mg; Vit. B1 = 1mg; Vit. B2 = 4mg; Vit. B6 = 4mg; Vit. B12 =16mg, Vit. C = 200mg; Niacin = 10mg; Folic acid =1.6mg; Biotin= 60mg; Mn = 80mg; Fe = 25mg; Cu = 5mg; Zn = 50mg; Se=200mg and KI=1.2mg.*

a = not fermented, b = fermented without S. cerevisiae, c = fermented with S. cerevisiae

5.3.6 Data collection

Feed intake

Every morning at 09:00hrs, the leftover feed was collected per cage of 6 chicks, weighed, and recorded before providing fresh feed. Daily feed intake was calculated as the amount of feed offered minus the amount of leftover feed.

Daily feed intake = feed offered – leftover feed

Bodyweight gain

The chicks within a cage were weighed once every week before feeding. Bodyweight gain (BWG) and average daily gain (ADG) per cage were calculated. BWG was calculated as final weight minus initial weight and ADG as the final weight minus initial weight divided by seven days.

Bodyweight gain= final weight- initial weight

$$Average\ daily\ gain = \frac{final\ weight - \ initial\ weight}{7}$$

Gain: feed ratio

Gain: feed ratio, a measure of the amount of feed required to attain one unit of weight gain was calculated as the average daily gain (ADG) divided by average daily feed intake (ADFI).

$$Gain{:}\ feed\ ratio = \frac{average\ daily\ gain}{average\ daily\ feed\ intake}$$

5.3.7 Statistical analysis

The data analysis of variance (ANOVA) was done using the General linear model (GLM) of Statistical Analysis Systems (SAS, 2009) software. The model was

$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$

where:

Y_{ijk} = observation associated with replication k of the factor combination ij

μ = overall population mean

α_i = effect of factor A (fermentation types)

β_j = effect of factor B (level of AFs)

$(\alpha\beta)_{ij}$ = interaction of factor A level i with B level j

ε_{ijk} = random error associated with Y_{ijk}

5.4 Results

The results of this experiment are summarised in Tables 5.2, 5.3, 5.4 and 5.5 below.

Table 5.2 Effect of diet on feed intake, body weight gain and gain: feed ratio of broiler chickens

Parameters	Means						SEM	*p*-value
	Diet1	Diet2	Diet3	Diet4	Diet5	Diet6		
Intake (g/d)	22.1	22.3	23.0	21.0	22.5	23.0	1.52	0.941
Body weight gain (g/d)	13.3	13.3	9.0	8.6	13.9	10.9	1.52	0.08
Gain: feed ratio	0.6	0.6	0.39	0.41	0.62	0.47	0.13	0.083

Diet 1: No aflatoxin and not fermented, Diet 2: No aflatoxin and fermented without S. cerevisiae, Diet 3: No aflatoxin and fermented with S. cerevisiae, Diet 4: Contained 30.08ppb aflatoxin and not fermented, Diet 5: Contained 30.08ppb aflatoxin and fermented without S. cerevisiae, Diet 6: Contained 30.08ppb aflatoxin fermented with S. cerevisiae.

The feed intake, body weight gain and gain: feed ratio was not improved by dietary treatment. The diet fermented with and without *S. cerevisiae* offered the same advantage on intake, body weight gain and gain: feed ratio within 21days.

Table 5.3 Effect of fermentation type and aflatoxins on feed intake (g/d), body weight gain (g/d) and gain: feed ratio of broiler chickens

Fermentation types

Parameters	Not fermented	Fermented without *S. cerevisiae*	Fermented with *S. cerevisiae*	*SEM*	*p*-value
Intake (g/d)	21.6	22.4	22.9	2.879	0.647
Body weight gain (g/d)	10.8	13.5	9.8	3.209	0.083
Gain: feed ratio	0.51^{ab}	0.60^{a}	0.43^{b}	0.131	0.048

Total aflatoxin levels

Parameters	0ppb	30.08ppb	*SEM*	*p*-value
Intake (g/d)	22.4	22.2	2.87	0.849
Body weight gain (g/d)	11.7	11.1	3.516	0.706
Gain: feed ratio	0.51	0.51	0.148	0.913

The feed intake of the non-contaminated and contaminated diets was not improved by fermentation (Table 5.3). Fermentation without *S. cerevisiae* and fermentation with *S. cerevisiae* had a similar effect on feed intake, whether the diet is contaminated or not. The fermentation types had no significant impact on the intake and body weight gain. The gain: feed ratio of chicken had been affected by fermentation types with *p*-value=0.048. The total aflatoxin levels had no significant effect on the chicken's intake, bodyweight gain and gain:feed ratio.

Table 5.4 Dietary effect on the mortality rate of broiler chickens after 21days

Diet	Number of chicks per diet	Number of dead chicks	Mortality rate (%)
Diet 1	24	1	4.2
Diet 2	24	3	12.5
Diet 3	24	4	16.7
Diet 4	24	18	75.0
Diet 5	24	7	29.2
Diet 6	24	14	58.3

Diet 1: No aflatoxin and not fermented, Diet 2: No aflatoxin and fermented without S. cerevisiae, Diet 3: No aflatoxin and fermented with S. cerevisiae, Diet 4: Contained 30.08ppb aflatoxin and not fermented, Diet 5: Contained 30.08ppb aflatoxin and fermented without S. cerevisiae, Diet 6: Contained 30.08ppb aflatoxin fermented with S. cerevisiae.

The mortality rate was very high 75%, in no-fermented contaminated feed during 21days feeding period. Fermentation with and without *S. cerevisiae* decreased the mortality rate in the contaminated diet.

5.5 Discussion

The feed intake, body weight gain and gain: feed ratio was not significantly ($p>0.05$) different within the six diets (Table 5.2). Fermentation types did not significantly improve the feed intake and body weight gain within the 21days but improved the gain: feed ratio (Table 5.3). The study done by Naji *et al.* (2016) indicated that fermented feed with probiotic was economically beneficial since it improved the broiler feed conversion ratio. Broiler chickens fed on moist fermented feed showed a detrimental effect on early bird growth but affected beneficially feed efficiency (Missotten *et al.,* 2013). A wet fermented feed with prepared probiotic caused a significant improvement in the chicken feed conversion ratio (Jawad *et al.,* 2016).

The total aflatoxin level of 30.08ppb had no significant effect on intake, body weight gain and feed conversion ratio (Table 5.3). Many studies reported the decrease in feed intake and body weight gain and an increase in feed conversion ratio when higher levels of aflatoxin were used in the feed (Table 5.5).

Table 5.5 Summary of different studies done on the effects of aflatoxin levels on body weight gain, feed intake and feed conversion ratio of broiler chicken.

Aflatoxin levels in the feed	Effects on body weight gain, feed intake, and FCR	References
700ppb, 1700ppb and 2800ppb AFs	Lowered body weights, depressed feed intake, increased FCR	(Marchioro *et al*., 2013)
1500ppb AFB1	Impaired growth	(Chen *et al.,* 2016)
1000ppb AFB1	Lowered growth rate	(Ali Rajput *et al.,* 2017)
200ppb and 400ppb AFs	Reduced body weight, daily weight gain, feed intake and increased FCR	(Valchev *et al.,* 2017)
40ppb and 80ppb AFB1	80ppb resulted in lower body weight gain and feed efficiency	(Denli and Okan, 2006)

The mortality rate was 75.0% (Table 5.4) in the non-fermented feed with 30.08ppb total aflatoxin level which was higher than the United States Food and Drug Administration (USFDA) and European Union Commission (EUC) maximum tolerable limit of 20ppb in poultry (Morrison *et al.,* 2017). Sobrane *et al.* (2016) observed the 20.13%±9.45 mortality rate of broilers fed contaminated feed with 2000ppb aflatoxin B1. Shivachandra *et al.*, 2003 recorded a mortality rate 56.7%, and reductions in body weight gain, in the organ to body weight ratios of the bursa and spleen in broiler chicks fed on feed with 1ppm aflatoxin level. Contamination of broiler chickens feed by AFB1 (2000ppb) caused liver impairment, weight loss, difficulty in protein synthesis, immune suppression, and anorexia, which exposed them to other challenges present in the rearing environment, thereby increasing mortality (Lopes *et al.,* 2006). During this study, some of the chicks fed on aflatoxins contaminated feed were not able to stand on their feet. Similar findings were reported by Khan *et al.,* (1990) where three strains of the broiler chicken, which were fed on a diet with 250ppb of aflatoxin contamination for three weeks, generally showed in some cases, raffled feathers and a few were not able to stand on their feet.

5.6 Conclusion

Fermentation with and without using *S. cerevisiae* (NCYC 125®) did not affect feed intake, body weight gain but improved gain: feed ratio of broiler chicken.

5.7 Recommendation

Further research should be conducted to determine the effect of fermented feed on the quality of meat.

5.8 References

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CHAPTER SIX
GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

Aflatoxins are mycotoxins produced by fungi, mainly *Aspergillus flavus* and *Aspergillus parasiticus* (De Oliveira *et al.,* 2018). They are responsible for significant hazards in commercial poultry health and livestock production, mainly due to financial losses resulting from the decrease in animal performance (Carão *et al.,* 2014). Feed quantity, quality, and palatability have been of considerable concern for a long time (Sugiharto, 2019). Among the factors affecting the quality of feed, feed contamination by the fungi has been reported to negatively influence the organoleptic properties and the quality of broiler feed (Greco *et al*., 2014). The fungi may assimilate, grow and utilise the readily available nutrients in broiler feed (Ghaemmaghami *et al.,* 2016). Because of the importance that mycotoxins pose to poultry production, it is necessary to adopt measures to prevent contamination, such as developing control programs to combat fungal growth and toxin production (De Oliveira *et al.,* 2018).

Fermentation, a popular method to improve the nutritional contents of feed is associated with a high number of lactic acid bacteria (LAB), a low pH and a high concentration of organic acids (Canibe and Jensen, 2012) and is the best method (Byakika *et al.,* 2019) to reduce aflatoxin levels in contaminated feed. During fermentation, lactic acid bacteria remove toxins through non-covalent binding of mutagens by fractions of the cell wall skeleton of the lactic acid bacteria. Another alternative mechanism of aflatoxin B1 removal has been reported in which lactic acid bacteria fermentation opens up the aflatoxin B1 lactone ring resulting in its complete detoxification (Zhang and Ohta 1991; Nout 1994). The current study found that during fermentation, the pH reduced from 6.9-5.0 and the total aflatoxins reduced by 52 and 53.4% within 72hours when fermented naturally and with yeast, respectively. These results showed that natural fermentation was as effective as fermentation with yeast. Fermentation showed a significant effect on dry matter digestibility and metabolizable nitrogen. Natural fermentation (fermentation without yeast) improved dry matter digestibility of the clean and aflatoxins contaminated diets. Fermentation did not improve feed intake and body weight gain. However, gain: feed ratio was better in broilers fed diets which were fermented without yeast. The mortality rate was very low in contaminated feed fermented naturally compared to that fermented with yeast.

6.2 General Conclusions

1. Fermentation with and without using *S. cerevisiae* (NCYC 125®) in a ratio of 1:1.5 (w/v) of the substrate to water for 72 hours reduced total aflatoxin contents by 53.4% and 52.0%, respectively.
2. Contaminated feed fermented without using *S. cerevisiae* (NCYC 125®) improved dry matter digestibility and metabolizable nitrogen of broiler chickens feed.
3. Contaminated feed fermented with and without using *S. cerevisiae* (NCYC 125®) has no effect on feed intake and growth of broiler chickens.

6.3 General Recommendations

1. There is a need for further research to investigate the best fermentation period (hours) of contaminated maize under different fermentation conditions and yeast strains.
2. Conduct further studies on using digestibility of nitrogen as a measure to find out the amount of total aflatoxins which does not cause injury of the pancreas
3. Further research should be conducted to determine the effect of fermented feed on the quality of meat.

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APPENDICES

Data for the first objective

Experiment one

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
Fermentation	2	1 2
Duration	6	1 2 3 4 5 6

Number of observations 36

Dependent Variable: Total aflatoxin levels

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	347.4000000	57.9000000	49.94	<.0001
Error	29	33.6200000	1.1593103		
Corrected Total	35	381.0200000			

R-Square	Coeff Var	Root MSE	Totalaflatoxinlevels Mean
0.911763	7.511949	1.076713	14.33333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Fermentation	1	0.2500000	0.2500000	0.22	0.6458
Duration	5	347.1500000	69.4300000	59.89	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Fermentation	1	0.2500000	0.2500000	0.22	0.6458
Duration	5	347.1500000	69.4300000	59.89	<.0001

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

Total aflatoxin levels Standard H0:LSMEAN=0 H0:LSMean1=LSMean2

Fermentation	LSMEAN	Error	Pr > |t|	t Value	Pr > |t|
1	14.4166667	0.2537836	<.0001	0.46	0.6458
2	14.2500000	0.2537836	<.0001		

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

Duration	Total aflatoxin levels LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	20.4000000	0.4395661	<.0001	1
2	15.6000000	0.4395661	<.0001	2
3	14.3000000	0.4395661	<.0001	3
4	11.2500000	0.4395661	<.0001	4
5	11.5000000	0.4395661	<.0001	5
6	12.9500000	0.4395661	<.0001	6

Least Squares Means for Effect Duration

t for H0: LSMean(i)=LSMean(j) / Pr > |t|

Dependent Variable: Total aflatoxin levels

i/j	1	2	3	4	5	6
1		7.721506	9.812747	14.71912	14.31696	11.98442
		<.0001	<.0001	<.0001	<.0001	<.0001
2	-7.72151		2.091241	6.997615	6.595453	4.262915
	<.0001		0.3195	<.0001	<.0001	0.0024
3	-9.81275	-2.09124		4.906374	4.504212	2.171674
	<.0001	0.3195		0.0004	0.0013	0.2811

4	-14.7191	-6.99761	-4.90637		-0.40216	-2.7347
	<.0001	<.0001	0.0004		0.9985	0.0986
5	-14.317	-6.59545	-4.50421	0.402162		-2.33254
	<.0001	<.0001	0.0013	0.9985		0.2139
6	-11.9844	-4.26291	-2.17167	2.7347	2.332538	
	<.0001	0.0024	0.2811	0.0986	0.2139	

Experiment two

The GLM Procedure

Class Level Information

Class	Levels	Values
Fermentation	2	1 2
Duration	9	1 2 3 4 5 6 7 8 9

Number of observations 54

The GLM Procedure

Dependent Variable: Total aflatoxin levels

Source	DF	Sum of Squares	Mean Square	F Value	Pr >F
Model	9	280.0855556	31.1206173	35.66	<.0001
Error	44	38.3937037	0.8725842		
Corrected Total	53	318.4792593			

R-Square	Coeff Var	Root MSE	Total aflatoxin levels Mean
0.879447	10.37487	0.934122	9.003704

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Fermentation	1	23.4696296	23.4696296	26.90	<.0001
Duration	8	256.6159259	32.0769907	36.76	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Fermentation	1	23.4696296	23.4696296	26.90	<.0001

Duration	8	256.6159259	32.0769907	36.76 <.0001

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

	Total aflatoxin levels	Standard	H0:LSMEAN=0	H0:LSMean1=LSMean2
Fermentation	LSMEAN	Error	Pr > |t|	t Value Pr > |t|
1	8.34444444	0.17977189	<.0001	-5.19 <.0001
2	9.66296296	0.17977189	<.0001	

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

	Totalaflatoxinlevels	Standard		LSMEAN
Duration	LSMEAN	Error	Pr > |t|	Number
1	14.3000000	0.3813538	<.0001	1
2	9.5666667	0.3813538	<.0001	2
3	7.7500000	0.3813538	<.0001	3
4	6.0500000	0.3813538	<.0001	4
5	7.3166667	0.3813538	<.0001	5
6	8.2500000	0.3813538	<.0001	6
7	8.7666667	0.3813538	<.0001	7
8	9.2833333	0.3813538	<.0001	8
9	9.7500000	0.3813538	<.0001	9

Least Squares Means for Effect Duration

t for H0: LSMean(i)=LSMean(j) / Pr > |t|

Dependent Variable: Totalaflatoxinlevels

i/j	1	2	3	4	5	6	7	8	9
1		8.776554	12.14502	15.29716	12.94851	11.21792	10.25992	9.301912	8.436617
		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
2	-8.77655		3.368466	6.520609	4.171954	2.441365	1.483361	0.525357	-0.33994

<.0001 0.0381 <.0001 0.0041 0.2883 0.8571 0.9998 1.0000

3 -12.145 -3.36847 3.152143 0.803487 -0.9271 -1.88511 -2.84311 -3.7084

<.0001 0.0381 0.0652 0.9962 0.9901 0.6270 0.1319 0.0154

4 -15.2972 -6.52061 -3.15214 -2.34866 -4.07924 -5.03725 -5.99525 -6.86055

<.0001 <.0001 0.0652 0.3369 0.0053 0.0003 <.0001 <.0001

5 -12.9485 -4.17195 -0.80349 2.348655 -1.73059 -2.68859 -3.6466 -4.51189

<.0001 0.0041 0.9962 0.3369 0.7249 0.1816 0.0182 0.0014

6 -11.2179 -2.44137 0.927101 4.079244 1.730588 -0.958 -1.91601 -2.7813

<.0001 0.2883 0.9901 0.0053 0.7249 0.9878 0.6068 0.1503

7 -10.2599 -1.48336 1.885105 5.037248 2.688592 0.958004 -0.958 -1.8233

<.0001 0.8571 0.6270 0.0003 0.1816 0.9878 0.9878 0.6670

8 -9.30191 -0.52536 2.843109 5.995252 3.646597 1.916008 0.958004 -0.86529

<.0001 0.9998 0.1319 <.0001 0.0182 0.6068 0.9878 0.9937

9 -8.43662 0.339937 3.708403 6.860546 4.511891 2.781302 1.823298 0.865294

<.0001 1.0000 0.0154 <.0001 0.0014 0.1503 0.6670 0.9937

Data for the second objective

The ANOVA Procedure

Class Level Information

Class	Levels	Values
Diet	6	1 2 3 4 5 6
Aflatoxins	2	0 28.4
Fermentation	3	0 1 2

Number of observations 24

Dependent Variable: DMD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	272.2916667	34.0364583	5.94	0.0016
Error	15	85.9745833	5.7316389		
Corrected Total	23	358.2662500			

R-Square	Coeff Var	Root MSE	DMD Mean

0.760026	3.594046	2.394084	66.61250	

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Diet	5	156.0987500	31.2197500	5.45	0.0047
Aflatoxins	1	8.5204167	8.5204167	1.49	0.2416
Fermentation	2	107.6725000	53.8362500	9.39	0.0023

Dependent Variable: ME

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	1276.398333	159.549792	Infty	<.0001
Error	15	0.000000	0.000000		
Corrected Total	23	1276.398333			

R-Square	Coeff Var	Root MSE	ME Mean
1.000000	0	0	18.35833

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Diet	5	869.1133333	173.8226667	Infty	<.0001
Aflatoxins	1	782.0416667	782.0416667	Infty	<.0001
Fermentation	2	53.6458333	26.8229167	Infty	<.0001

Dependent Variable: MN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	2190.446250	273.805781	Infty	<.0001
Error	15	0.000000	0.000000		
Corrected Total	23	2190.446250			

R-Square	Coeff Var	Root MSE	MN Mean
1.000000	0	0	42.71250

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Diet	5	1442.963750	288.592750	Infty	<.0001
Aflatoxins	1	928.770417	928.770417	Infty	<.0001
Fermentation	2	311.552500	155.776250	Infty	<.0001

Tukey's Studentized Range (HSD) Test for DMD

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type

II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	5.731639
Critical Value of Studentized Range	4.59474
Minimum Significant Difference	5.5001

Means with the same letter are not significantly different.

Tukey Grouping			Mean	N	Diet
	A		69.875	4	4
B	A		69.125	4	2
B	A	C	68.225	4	5
B	A	C	65.050	4	1
B		C	63.875	4	3
		C	63.525	4	6

Tukey's Studentized Range (HSD) Test for ME

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	0
Critical Value of Studentized Range	4.59474
Minimum Significant Difference	0

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Diet
A	26.20	4	2
B	25.50	4	3
C	20.50	4	1

D	13.93	4	6
E	12.05	4	4
F	11.98	4	5

Tukey's Studentized Range (HSD) Test for MN

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	0
Critical Value of Studentized Range	4.59474
Minimum Significant Difference	0

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Diet
A	50.28	4	6
B	48.30	4	5
C	48.23	4	4
D	43.05	4	3
E	38.75	4	2
F	27.68	4	1

Tukey's Studentized Range (HSD) Test for DMD

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	5.731639
Critical Value of Studentized Range	3.01432
Minimum Significant Difference	2.0832

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Aflatoxins

A	67.2083	12	28.4
A	66.0167	12	0

Tukey's Studentized Range (HSD) Test for ME

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	0
Critical Value of Studentized Range	3.01432
Minimum Significant Difference	0

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Aflatoxins
A	24.07	12	0
B	12.65	12	28.4

Tukey's Studentized Range (HSD) Test for MN

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	0
Critical Value of Studentized Range	3.01432
Minimum Significant Difference	0

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Aflatoxins
A	48.93	12	28.4
B	36.49	12	0

Tukey's Studentized Range (HSD) Test for DMD

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher

Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	5.731639
Critical Value of Studentized Range	3.67338
Minimum Significant Difference	3.1093

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Fermentation
A	68.675	8	1
A	67.463	8	0
B	63.700	8	2

Tukey's Studentized Range (HSD) Test for ME

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	0
Critical Value of Studentized Range	3.67338
Minimum Significant Difference	0

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Fermentation
A	19.71	8	2
B	19.09	8	1
C	16.28	8	0

Tukey's Studentized Range (HSD) Test for MN

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05

Error Degrees of Freedom	15
Error Mean Square	0
Critical Value of Studentized Range	3.67338
Minimum Significant Difference	0

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Fermentation
A	46.66	8	2
B	43.53	8	1
C	37.95	8	0

Data for the third objective

The GLM Procedure

Class Level Information

Class	Levels	Values
Diet	6	1 2 3 4 5 6

Number of observations 24

Dependent Variable: intake

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	10.8892109	2.1778422	0.24	0.9410
Error	18	165.3941482	9.1885638		
Corrected Total	23	176.2833592			

R-Square	Coeff Var	Root MSE	intake Mean
0.061771	13.56451	3.031264	22.34703

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Diet	5	10.88921094	2.17784219	0.24	0.9410

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Diet	5	10.88921094	2.17784219	0.24	0.9410

Dependent Variable: growth

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	108.9526416	21.7905283	2.37	0.0805
Error	18	165.2542619	9.1807923		
Corrected Total	23	274.2069035			

R-Square	Coeff Var	Root MSE	growth Mean
0.397337	26.30146	3.029982	11.52021

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Diet	5	108.9526416	21.7905283	2.37	0.0805

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Diet	5	108.9526416	21.7905283	2.37	0.0805

Adjustment for Multiple Comparisons: Tukey

Diet	Intake LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	22.1254875	1.5156322	<.0001	1
2	22.3336905	1.5156322	<.0001	2
3	23.0265079	1.5156322	<.0001	3
4	21.0405556	1.5156322	<.0001	4
5	22.5200000	1.5156322	<.0001	5
6	23.0359127	1.5156322	<.0001	6

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

Least Squares Means for effect diet

Pr > |t| for H0: LS Mean (i) =LSMean(j)

Dependent Variable: intake

i/j	1	2	3	4	5	6
1	1.0000	0.9980	0.9953	1.0000	0.9979	

2	1.0000	0.9994	0.9894	1.0000	0.9994
3	0.9980	0.9994	0.9344	0.9999	1.0000
4	0.9953	0.9894	0.9344	0.9808	0.9332
5	1.0000	1.0000	0.9999	0.9808	0.9999
6	0.9979	0.9994	1.0000	0.9332	0.9999

Diet	Growth LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	13.2996032	1.5149911	<.0001	1
2	13.3343254	1.5149911	<.0001	2
3	9.0454365	1.5149911	<.0001	3
4	8.6170635	1.5149911	<.0001	4
5	13.9349206	1.5149911	<.0001	5
6	10.8898810	1.5149911	<.0001	6

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

Least Squares Means for effect diet

Pr > |t| for H0: LSMean (i) =LSMean(j)

Dependent Variable: growth

i/j	1	2	3	4	5	6
1	1.0000	0.3873	0.2913	0.9996	0.8648	
2	1.0000	0.3789	0.2842	0.9997	0.8579	
3	0.3873	0.3789	0.9999	0.2511	0.9511	
4	0.2913	0.2842	0.9999	0.1812	0.8904	
5	0.9996	0.9997	0.2511	0.1812	0.7145	
6	0.8648	0.8579	0.9511	0.8904	0.7145	

Dependent Variable: Gain: feed ratio

The ANOVA Procedure

Class Level Information

Class	Levels	Values
Diet	6	1 2 3 4 5 6

Number of observations 24

The ANOVA Procedure

Dependent Variable: **Gain: feed ratio**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.19093969	0.03818794	2.35	0.0832
Error	18	0.29293371	0.01627410		
Corrected Total	23	0.48387341			

R-Square	Coeff Var	Root MSE	Gain: feed ratio Mean
0.394607	24.98790	0.127570	0.510527

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Diet	5	0.19093969	0.03818794	2.35	0.0832

The ANOVA Procedure

t Tests (LSD) for Gain: feed ratio

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	0.016274
Critical Value of t	2.10092
Least Significant Difference	0.1895

Means with the same letter are not significantly different.

t Grouping	Mean	N	Diet
A	0.61909	4	5
A	0.58527	4	1

	A	0.57889	4	2
B	A	0.47287	4	6
B	A	0.42967	4	4
B		0.37737	4	3

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for Gain: feed ratio

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	0.016274
Critical Value of Studentized Range	4.49442
Minimum Significant Difference	0.2867

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Diet
A	0.61909	4	5
A	0.58527	4	1
A	0.57889	4	2
A	0.47287	4	6
A	0.42967	4	4
A	0.37737	4	3

Publication abstract

The effect of fermentation with and without *Saccharomyces cerevisiae* on the levels of aflatoxin in maize

Mukandungutse B I, J K Tuitoek, A M King'ori and M A Obonyo[1]
Department of Animal Sciences, Egerton University, P O Box 536 - 20115, Egerton, Kenya
muisabetty@gmail.com
[1]Department of Biochemistry& Molecular Biology, Egerton University, PO Box 536 - 20115, Egerton

https://www.lrrd.org/public-lrrd/proofs/lrrd3111/muisa31172.html

Abstract

Aflatoxins occur as natural contaminants in cereals. Poultry feeds are mainly formulated using maize because of its high metabolizable energy. It has been suggested that fermentation can reduce levels of aflatoxins in contaminated maize. This study evaluated the effect of fermentation, under different conditions, on aflatoxin levels in contaminated maize flour. Clean (aflatoxin-free) maize was moistened in water and inoculated with *Aspergillus flavus* then incubated at 30°C for 60 days with frequent moistening. In the first and second experiments, maize flour was fermented with and without yeast *Saccharomyces cerevisae* between 5-8 days. In the third experiment, the flour to water ratio was adjusted from 1:1 to 1:1.5 and fermented for 72hours. During fermentation there was reduction in pH (6.9-5.0) as well as total aflatoxins by 52 and 53.4% when fermented either naturally or with yeast respectively. The final Aflatoxin levels reduced from 20.4ppb (unsafe for poultry) to 10.9ppb (safe) level. However, the level of aflatoxins steadily increased when fermentation proceeded after 72hours. These results show that natural fermentation (without yeast) was as effective as that with yeast since there were no significant difference in percentage of aflatoxin reduction. It is concluded that the best ratio of flour to water for fermentation was 1:1.5 and fermentation period of 72hours. Therefore, this simple method of fermentation contributed to reduced aflatoxin levels in flour for poultry feed production. A prerequisite would be to carry out organoleptic tests and proximate analysis of feed material.

Key words: *Feed, Natural fermentation, Poultry*

National Commission for Science, Technology and Innovation Research permit

THIS IS TO CERTIFY THAT:
MS. ISABELLE BEATRICE MUKANDUNGUTSE
of EGERTON UNIVERSITY, S36-20115
NAKURU,has been permitted to conduct research in *Nakuru County*

on the topic: *DETOXIFICATION OF AFLATOXINS IN BROILER CHICKEN FEEDS BY FERMENTATION*

for the period ending:
23rd May,2020

Permit No : NACOSTI/P/19/17050/30200
Date Of Issue : 27th May,2019
Fee Recieved :Ksh 1000

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Applicant's Signature

.....
Director General
National Commission for Science, Technology & Innovation