بسم الله الرحمن الرحيم

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تم عمل المسح الضوئي لهذة الرسالة بواسطة / حسام الدين محمد مغربي

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

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MYCOLOGICAL EXAMINATION OF POULTRY FEED STUFF WITH SPECIAL REFERENCE TO MYCOTOXIN PRODUCTION

Thesis Presented

By

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قرار لجنة الحكم و المناقشة

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CONTENTS

Introduction

1.0 INTRODUCTION

Fungi and mycotoxins in poultry feeds and feed ingredients attracted much attention during the last decade as a result of increasing awareness of health hazards presented by those fungi and their toxins ***(Hsieh, 1990b)***. Mycotoxins cause severe economic losses in poultry and livestock industries. In many cases, aflatoxin contamination may mean difference between profit and loss to the poultry industry ***(Kubena et al., 1998)***. Fungi that produce mycotoxins are widely distributed in feeds and feed ingredients contaminated with these mycotoxins produced by fungi that are grown on crops in the field after harvest, in storage or during processing of food and feed stuffs ***(Clark et al., 1980)***. Almost all plant products can serve as substrates for fungal growth and subsequent mycotoxin formation. This provides the potential for direct contamination of food, animal feed and poultry feed ***(Shotwell et al., 1966)***. Among many of discovered mycotoxins, aflatoxins, ochratoxin A and several others were identified ***(Richard et al., 1993)***.

Aflatoxin is the most commonly encountered mycotoxin in feed stuffs ***(Richard et al., 1993)***. Aflatoxin is a group of closely related toxic and carcinogenic metabolites mainly produced by *Aspergillus flavus, Aspergillus parasiticus* and *Penicillium puberulum* in the field and during storage in a number of important agricultural commodities such as grains and feed ***(Edds, 1979; Clark et al., 1980)***. Inadequate storage conditions such as high moisture and warm temperature (25 – 30 °C) can create conditions suitable for aflatoxin production ***(Cast, 1989)***.

Feeds which are contaminated with mycotoxins not only have a direct toxic effect on animal or poultry but there may be a carry over of the toxin into the product creating further exposure of human beings to this intoxication ***(Van Zytveld et al., 1970*** and ***Mabee and Chipley, 1973)***. Aflatoxin containing feed lead to aflatoxicosis ***(Shotwell et al., 1966)***. Aflatoxin is the most toxic ***(Wogan, 1973)***, mutagenic ***(Chu, 1991)***, immunosuppressive ***(Thurston et al., 1986*** and ***Cusumano et al., 1990)*** and teratogenic agent ***(Awad et al., 1989*** and ***Abou El-Magd, 1992)***.

The discovery of aflatoxin B1 (AFB1) as one of the most potent naturally occurring carcinogenic substance in the early 1960's generated considerable researches in this area ***(Busby and Wogan, 1981*** and ***Cole and Cox, 1981)***.

Ochratoxins are among the most toxic mycotoxins to poultry. Ochratoxins are nephrotoxic metabolites produced chiefly by *Penicillium viridicatum* and *Aspergillus ochraceus* which commonly occur on numerous grains and feed stuffs ***(Dwivedi and Burns, 1986)***. Ochratoxins are designated as A, B, C, D. Ochratoxin A is the most common and most toxic and relatively stable. Some ochratoxin-producing fungi produce other mycotoxins toxic to poultry including citrinin. Ochratoxin A was first identified in the feed chain in 1969 as a contaminant of corn then has been found in a variety of grains including wheat and barley ***(Carlton and Korgh, 1979)***.

Analytical techniques for mycotoxins are include chromatography (thin layer, gas, liquid), mass spectrophotometry and monoclonal

antibody based technology. The black-liquid evaluation of grains for *Aspergillus flavus* growth is an acceptable presumptive test for aflatoxin, but does not confirm presence of actual toxin ***(Bathast and Hesseltine, 1975)***.

So the aim of this study is the determination of the incidence of mycotoxin contaminated samples and fungi producing it in suspected samples and trying to overcome the problem according to the results and interpretation of the results through investigating the following points:

1. Isolation and identification of mycotoxin producing fungi.
2. Quantification of specific toxins using monoclonal-antibody technique using flurometer (ViCAM V series 4).
3. Detection of aflatoxin-producing ability of *Aspergillus flavus* isolates.

Review of Literature

2.0 REVIEW OF LITERATURE

2.1. Aflatoxins:

2.1.1. Occurrence of aflatoxins:

The aflatoxin-producing mold *Aspergillus flavus* is most likely to occur at harmful levels in grains and seeds that have been stored too wet for an extended period but may under certain conditions, develop prior to harvest.

Corn received the greatest attention as a possible aflatoxin source but barley, grain sorghum, wheat, cotton seed, peanuts and soya beans, as well as processed feeds produced from these grains and seeds have sometimes shown to contain high levels of aflatoxin.

The aflatoxins are recognized as being widely distributed in general, *A. flavus* and *A. parasiticus* are capable of producing aflatoxin on many feed or food ingredients.

Boudergues et al. (1966) estimated the aflatoxin content of leaf, stem, shell and whole dried haulms of ground nuts used for livestock feeding in Dakar. The whole straw had 311 ppm AFB and 6 ppm AFG; leaves had 3 and 18; stems 0.6 and 0.9 and shells 3 and 3 ppm, respectively.

Lucas et al. (1970) reported that 3% of the examined Vietnam rice samples were positive for aflatoxin.

Shotwell et al. (1970) examined more than 500 samples of maize for aflatoxin contamination. They reported that 3% of the samples contained aflatoxin in a range of 3 – 37 ppb.

Albert et al. (1971) evaluated corn contamination with aflatoxin B1 and revealed its occurrence in 35 – 40% of the total samples examined with an average level of 213 ppb.

Shotwell et al. (1973) examined corn samples for aflatoxin contamination and revealed the detection of B1, B2, G1 and G2 at percentage of 35, 25, 8.33 and 3.33, respectively of the total samples with a range level of 6 – 308 ppb.

Frosile and Wassijo (1974) studied the contents of aflatoxins in 86 samples of ground nut meal imported into Norway during 1968 – 1973. They found that 19.8% had <100, 41.8% had 100 – 500, 26.8% had 500 – 1000 and 11.6% had >1000 µg aflatoxin/kg. There were marked differences between countries of origin but small variation was noticed between years.

Hesseltine (1979) stated that aflatoxins were found in rice of the following countries as follows in less than 1 ppb in Mozambique, 16 ppb in Philippines, 180 – 220 ppb in Taiwan, 98 ppb in Thailand and 282 ppb in USA.

Strzelick and Gasiorowaska (1974) examined 306 samples of feed stuffs and concentrates for aflatoxin contamination. They showed that 12.7% were contaminated with aflatoxin B1 at level above the permissible limit (0.2 – 0.3 ppm).

Detory et al. (1977) observed that aflatoxin contamination was found to be higher in some crops than others it can be arranged as

follows: coconut > rice = wheat cotton (plants) > dats > peanut > soya bean > clover.

Josefesson and Moller (1977) screened cereal samples for mycotoxin contamination. They revealed that the lowest levels of detected aflatoxin was 5 µg/kg in corn and 10 µg/kg in wheat.

Pensala et al. (1977) examined nut products imported into Finland during the years 1974 – 1976 for aflatoxin contamination. 4.2% were found to contain AFB1 and B2 especially in cracked or sliced kernels. The aflatoxin content was less than 6 ng/g in 20.5%; 6 – 10 ng/g in 52.3%101 – 500 ng/g in 20.5% and more than 500 ng/g in 6.7% in samples positive for aflatoxin.

In Egypt, ***Girgis et al. (1977)*** reported that in each of 6 samples of wheat and corn collected from various localities in Egypt, aflatoxin B1, B2, G1 and G2 were detected at levels of 10 ppb or more. They also obtained aflatoxin in 16.7% of bean samples at levels of 17 ppb for B1 and 1.5 ppb for B2. They also analyzed six samples of cotton seed cake and found that aflatoxin B1, B2, G1 and G2 revealed a range of 10 ppb to 117 ppb.

Chelkowski et al. (1978) studies the occurrence of aflatoxin in some suspected samples of polish grain and imported meal seeds taken during the years of 1970 – 1976. Out of 21 samples of wheat contained AFB1 at a concentration of 8.4 µg/kg. Moreover, 1 barley sample out of 24 had AFB1 at a level of 3.4 µg/g at all 14 examined samples of ground nut meal contained AFB1 at a level of about 1 µg/kg.