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# **Rapid immunological assays for detection of aflatoxin B<sub>1</sub> in animal feed and its products**

Thesis presented by

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(Bacteriology, Mycology and Immunology)**

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## **ABSTRACT**

Fungi and their toxins are extensively widespread as those fungi are ubiquitous in nature. Aflatoxin B<sub>1</sub>, a fungal secondary metabolite excreted mainly by toxigenic *Aspergillus flavus*, considered as the most dangerous naturally occurring toxin, has carcinogenic effect on both human and animals. Aflatoxin B<sub>1</sub> was successfully produced from local toxigenic *Aspergillus flavus* strain on YES medium and its amount was determined using HPLC. Aflatoxin B<sub>1</sub> is a toxic fungal metabolite of low molecular weight, and hence is devoid of antigenicity. Those toxins also lack a reactive group for coupling with a macromolecule carrier for antibody production. Therefore, the main goal of this study was to produce polyclonal antibody (IgG) against aflatoxin B<sub>1</sub> through immunization of New Zealand white rabbits by the prepared antigen (AFB<sub>1</sub>-BSA Conjugate). The produced polyclonal antibodies were used in the production of a diagnostic product that was tested against spiked and naturally contaminated samples. Spiked samples were tested using both sandwich and competitive ELISA. In case of sandwich ELISA, the recovery % obtained from finished cattle feed samples ranged from 80.3% and 96% while in meat samples AFB<sub>1</sub> ranged from 78.1% and 93%. For competitive ELISA, the mean recovery % of AFB<sub>1</sub> obtained from finished cattle feed samples ranged from 88.7% and 97% , while in meat samples it ranged from 89.6% and 103%. Using competitive ELISA, The produced product gave promising results and was efficient in detecting AFB<sub>1</sub> in the examined naturally contaminated samples. In conclusion, the diagnostic product produced in the present study is simple and economic. ELISA procedure that can be used to screen aflatoxin B<sub>1</sub> in variety of samples (animal feed samples, meat and meat products). Thus, it is possible to screen large number of samples simply and inexpensively for the presence of aflatoxin B<sub>1</sub>.

**Key words: Aflatoxin B<sub>1</sub>, HPLC, Polyclonal antibody, ELISA, TLC, Animal feed, Meat, Meat products.**



*Dedicated to*  
*My beloved parents,*  
*my dear brothers,*  
*my sincere husband*  
*and my lovely son*



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# LIST OF CONTENTS

	<b>Page</b>
<b>I. INTRODUCTION</b> .....	1
<b>II. REVIEW OF LITERATURE</b> .....	5
1. Aflatoxins.....	5
2. Aflatoxin producing molds in animal feed, meat and meat products.....	12
2.1. Aflatoxin producing molds in animal feed.....	12
2.2. Aflatoxin producing molds in meat and meat products.....	16
3. Aflatoxins in animal feed, meat and meat products.....	20
3.1. Aflatoxins in animal feed.....	20
3.2. Aflatoxins in meat and meat products.....	31
4. Production of aflatoxin B <sub>1</sub> from toxigenic <i>Aspergillus flavus</i> strain.....	35
5. Preparation of the AFB <sub>1</sub> oxime and their protein conjugate.....	39
6. Polyclonal antibody production against AFB <sub>1</sub> and its application.....	43
7. Detection of aflatoxin B <sub>1</sub> using enzyme linked immunosorbent assay (ELISA).....	58
<b>III. MATERIAL AND METHODS</b> .....	65
<b>1. MATERIAL</b> .....	65
1.1. Materials used for identification of <i>Aspergillus flavus</i> .....	65
1.2. Materials used for production of aflatoxin B <sub>1</sub> .....	67
1.3. Materials used for HPLC-FLD.....	67
1.4. Materials used for conjugation of aflatoxin B <sub>1</sub> with bovine serum albumin.....	68
1.5. Materials used for production of polyclonal antibodies against aflatoxin B <sub>1</sub> .....	69
1.6. Materials used detection and evaluation of the produced polyclonal antibodies.....	69
1.7. Other materials.....	73
<b>2. METHODS</b> .....	75
1. Microscopical examination of <i>Aspergillus flavus</i> .....	75
2. Microscopical examination of <i>Aspergillus flavus</i> .....	75
3. Production of aflatoxin B <sub>1</sub> .....	76
4. Polyclonal antibody production against AFB <sub>1</sub> .....	78
5. Evaluation of the ability of the produced antibodies to detect aflatoxin B <sub>1</sub> in spiked samples using Sandwich ELISA.....	82
6. Evaluation of the efficacy of the produced diagnostic product to detect aflatoxin B <sub>1</sub> in spiked and naturally contaminated samples using competitive ELISA.....	85
7. Calculations.....	91
8. Statistical analysis.....	91
<b>IV. RESULTS</b> .....	93
<b>V. DISCUSSION</b> .....	126
<b>VI. COCLUSION</b> .....	141
<b>VII. SUMMARY</b> .....	142
<b>VIII. REFERENCES</b> .....	148
<b>ARABIC SUMMARY</b> .....	

## LIST OF TABLES

<b>Table no.</b>	<b>Title</b>	<b>Page</b>
<b>(1)</b>	Antibody titre (units/ml) obtained after injection with the prepared AFB <sub>1</sub> -BSA using indirect ELISA.	<b>101</b>
<b>(2)</b>	Mean recovery percentage and CV% of AFB <sub>1</sub> from spiked finished cattle feed samples using the prepared anti AFB <sub>1</sub> polyclonal antibodies by sandwich ELISA.	<b>106</b>
<b>(3)</b>	Mean recovery percentage and CV% of AFB <sub>1</sub> from spiked meat samples using the prepared anti AFB <sub>1</sub> polyclonal antibodies by sandwich ELISA.	<b>108</b>
<b>(4)</b>	Mean recovery percentage and CV% of AFB <sub>1</sub> from spiked finished cattle feed samples using the prepared anti AFB <sub>1</sub> polyclonal antibodies by competitive ELISA.	<b>111</b>
<b>(5)</b>	Mean recovery percentage and CV% of AFB <sub>1</sub> from spiked meat samples using the prepared anti AFB <sub>1</sub> polyclonal antibodies by competitive ELISA.	<b>113</b>
<b>(6)</b>	Aflatoxin B <sub>1</sub> concentration (ppb) in the positive naturally contaminated examined animal feed, meat and meat product samples using TLC technique.	<b>116</b>
<b>(7)</b>	AFB <sub>1</sub> concentration (ppb) in positive naturally contaminated samples using the prepared polyclonal antibody in competitive ELISA.	<b>119</b>

<b>(8)</b>	Comparison between the mean concentration values of positive results obtained from both TLC and the produced diagnostic product in detection of the concentration of AFB <sub>1</sub> in naturally contaminated samples.	<b>121</b>
<b>(9)</b>	Collective results of both TLC and ELISA in terms of Max., Min., Mean $\pm$ SE in detection of AFB <sub>1</sub> concentration in naturally contaminated samples.	<b>122</b>
<b>(10)</b>	Calculation of sensitivity, specificity, positive predictive value, negative predictive value and diagnostic efficiency.	<b>124</b>

## LIST OF FIGURES

Figure no.	Title	Page
(1)	Chemical structure of main aflatoxins divided into two groups, B and G family.	11
(2)	Schematic diagram for the production of BSA-aflatoxin B <sub>1</sub> -oxime conjugate.	40
(3)	High performance Liquid Chromatogram of aflatoxins standards (AFG <sub>1</sub> , AFB <sub>1</sub> , AFG <sub>2</sub> and AFB <sub>2</sub> ).	98
(4)	High performance Liquid Chromatogram of aflatoxins (AFG <sub>1</sub> , AFB <sub>1</sub> and AFB <sub>2</sub> ) of the chloroform extract of toxigenic <i>A. flavus</i> .	99
(5)	Anti aflatoxin B <sub>1</sub> titre (units/ml) at different weeks post AFB <sub>1</sub> -BSA injection.	102
(6)	Standard curve from OD values and the concentration of AFB <sub>1</sub> standard (ppb) using sandwich ELISA for AFB <sub>1</sub> detection.	104
(7)	Mean recovery percentage of AFB <sub>1</sub> from spiked finished cattle feed samples using the prepared anti AFB <sub>1</sub> polyclonal antibodies by sandwich ELISA.	107
(8)	Mean recovery percentage of AFB <sub>1</sub> from spiked meat samples using the prepared anti AFB <sub>1</sub> polyclonal antibodies using sandwich ELISA.	109

<b>(9)</b>	Standard curve from OD values and the concentration of AFB <sub>1</sub> standard (ppb) using competitive ELISA for AFB <sub>1</sub> detection.	<b>110</b>
<b>(10)</b>	Mean recovery percentage of AFB <sub>1</sub> from spiked finished cattle feed samples using the prepared anti AFB <sub>1</sub> polyclonal antibodies using competitive ELISA.	<b>112</b>
<b>(11)</b>	Mean recovery percentage of AFB <sub>1</sub> from spiked meat samples using the prepared anti AFB <sub>1</sub> polyclonal antibodies using competitive ELISA.	<b>114</b>
<b>(12)</b>	Percentage of positive aflatoxin B <sub>1</sub> naturally contaminated samples in the examined animal feed, meat and meat product samples using TLC technique.	<b>117</b>
<b>(13)</b>	Comparison between the mean concentration values of positive results obtained from both TLC and the produced diagnostic product in detection of the concentration of AFB <sub>1</sub> in naturally contaminated samples.	<b>123</b>
<b>(14)</b>	Collective results of testing sensitivity, specificity, predictive values and diagnostic efficiency of the prepared diagnostic product.	<b>125</b>

## LIST OF PHOTOS

<b>Photo no.</b>	<b>Title</b>	<b>Page</b>
<b>(1)</b>	<i>A. flavus</i> colonies on PDA (ten days old).	<b>94</b>
<b>(2)</b>	Reverse side of <i>A. flavus</i> colonies on PDA (ten days old).	<b>94</b>
<b>(3)</b>	The yellowish green mycelial growth of <i>A. flavus</i> on YES medium (15 days old).	<b>94</b>
<b>(4)</b>	The microscopical appearance showing typical heads of <i>A. flavus</i> stained with lactophenol cotton blue stain (40 X).	<b>94</b>
<b>(5)</b>	The spots of AFB <sub>1</sub> in both standard and the extracted samples in TLC plate under UV.	<b>96</b>
<b>(6)</b>	The spots of the purified AFB <sub>1</sub> under UV.	<b>96</b>
<b>(7)</b>	The spots of standard aflatoxins and naturally contaminated samples in TLC plate under UV.	<b>115</b>

## **LIST OF ABBREVIATIONS**

A.	Aspergillus
Ab	Antibody
AFB <sub>1</sub>	Aflatoxin B <sub>1</sub>
AFLA	Aflatoxin
AFM <sub>1</sub>	Aflatoxin M <sub>1</sub>
Ag	Antigen
a <sub>w</sub>	Water activity
BSA	Bovine serum albumin
cfu/g	Colony forming unit per gram
conc.	Concentration
CV	Coefficient of variation
Dis.	Distilled
DON	DeOxyNivalenol
EDPC	1- Ethyl - 3 (3- dimethyl aminopropyl) carbodiimide
ELISA	Enzyme Linked Immunosorbent Assay
fn	False-negative values
fp	False-positive values
F.	Fusarium
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
fig.	Figure
FUMB <sub>1</sub>	Fumonisin B <sub>1</sub>
gm	Gram
HRP	Horseradish peroxidase
HPLC-FLD	High Performance Liquid Chromatography with Fluorescence detector
IC <sub>50</sub>	Half maximal inhibitory concentration
IgG	Immunoglobulin G
IC - ELISA	Indirect competitive ELISA
Kg.	Kilogram
L	Liter
LOD	Limit of detection
LPCB	Lactophenol cotton blue

MeOH	Methanol water
Max.	Maximum
Min.	Minimum
mg.	Milligram
µg.	Microgram
ml	Milliliter
mM	Millimolar
MTLs	Maximum tolerated levels
NB	Nota bene
ND	Not detectable
nm	nanometer
NMR	Nuclear magnetic resonance
No.	Number
OPD	Ortho-phenylenediamidine
OD	Optical density
OTA	Ochractoxin A
P.	Penicillium
pAbs	Polyclonal antibodies
PBS	Phosphate buffer saline
PDA	Potato dextrose agar
pH	Hydrogen ion concentration
ppb	Parts-per billion
pps	Phosphate buffered saline
SDA	Sabouraud dextrose agar
SE	Standard error
spp.	Species
T-2	Trichothecene toxin
TLC	Thin layer chromatography
tn	True-negative values
tp	True-positive values
UV	Ultra violet
WHO	World Health Organization
YES	Yeast extract sucrose media
ZON	Zearalenone