

**The Biological And Chemical Evaluation Of Aflatoxin
Contaminated Ration After Chemical Treatment**

رسالة

BY

Mona Mohamed Abdel Galil

A thesis submitted in partial fulfillment

of

the requirements for the degree of

636-084
M-M



DOCTOR OF PHILOSOPHY

in

Agriculture Science

(Animal Nutrition)

49621

Departement of Animal Production

Faculty of Agriculture

Ain Shams University



1993

APPROVAL SHEET

The Biological And Chemical Evaluation Of Aflatoxin Contaminated Ration After Chemical Treatment

BY

Mona Mohamed Abdel Galil

B.Sc., Sci., (Animal Production), 1978, Cairo University

M.Sc., Agric. Sci., (Animal Nutrition), 1987, Ain Shams University

This thesis for Ph.D. degree has been

approved by:

Prof.Dr.M.Hathot.....*Mostafa Hathout*.....

Senior researches of Animal Nutrition Animal Production Institute
Research Ministry of Agriculture.

Prof.Dr.H.Khatab.....*Hamdi Khatab*.....

Prof. of Animal Nutrition Fac. of Agric., Ain Shams University.

Prof.Dr.M.A.El.Ashry.....*M.A. El Ashry*.....

Prof. of Animal Nutrition Fac. of Agric., Ain Shams University.

Date of examination: 29 / 7 / 1993



THE BIOLOGICAL AND CHEMICAL EVALUATION
OF AFLATOXIN CONTAMINATED RATIONS
AFTER CHEMICAL TREATMENT

By

MONA MOHAMED ABDEL-GALIL

B.Sc. Agric. (Animal Production), Cairo Univ., 1978
M.Sc. Agric. (Animal Production), Ain Shams Univ., 1987

Under the Supervision of:

Prof. M.A. El-Ashry
Prof. of Animal Nutrition
Head of Animal Production Dept.,
Fac. Agric., Ain Shams Univ.

Prof. Dr. Kh.M. Naguib
Prof. Of Mycotoxins
National Research Centre

Dr. H.M. Gado
Dr. of Animal Nutrition Dept.,
Fac. Agric., Ain Shams Univ.

ABSTRACT

Artificially contaminated corn containing 200 µg/kg total aflatoxins was treated with ammonia solution under varying ammonia concentrations (0.5, 1.0, 1.5 and 2.0%), moisture contents (18% added), different layer thickness of corn (1, 2, 3 and 4 cm), four different temperatures (25, 40, 50 and 60°C), different time of ammoniation (6, 24, 36 and 48 hr), and as well different times of agitation (6, 8 and 12 hr). Results indicate that total aflatoxin content of artificially contaminated corn was reduced from 200 to 15 µg/kg.

Clinicopathologic changes included decreased serum total protein (TP), increases serum bilirubin concentration, and alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Key Words

Aflatoxin, Ammonia, Corn, Goats.

ACKNOWLEDGEMENT

Firstly, thanks forever to **ALLAH** who is always helping me, as he helps all who research for the truth, because he is the truth.

To the eminent **Prof. Dr. M. Abdel-Moneam Elashry**, Prof. of Animal Production Department, and Head of Animal Production Department, Faculty of Agriculture, Ain Shams Univeristy, I would like to express my infinite gratitude. He supervised this work with unfailling, meticulous suggestions and honest assistance. It is his constructive criticism, scientific guidance and untiring help that made this work possible.

I would like to express my sincere gratitude for the kindness and encouragement of **Prof. Dr. Khayria M. Naguib**, Prof. of Mycotoxins and Head of Mycotoxins Central Lab., National Research Centre, under whose stimulating supervision, guidance and criticism this work was carried out and I heartly thanks very much for her interest so as to complete this thesis as precisely as it is.

I wish to express the sincerest gratitude to **Dr. Hany M. Gado**, Lecturer at Animal Production, Departement of Aminoal Production, Faculty of Agriculture, Ain Shams University, for his generous and kind help throughout this study.

It is a great pleasure to sincere gratitude for the kindnees to **Dr. Elham El Zanaty**, Prof. of Chemical Engineening, National Research Centre, for her fruitable help and guidance throughout the work of this thesis.

I'm really feel thankful and record my gratefull acknowledgement to staff members of Mycotoxins Central Lab.

C O N T E N T S

	Page
REVIEW OF LITERATURE	5
Part I - Detoxification	5
1. Detoxification by Physical Methods	5
1.1. Separation	5
1.1.1. Mechanical Separation	5
1.1.2. Density Segregation	6
1.2. Thermal Inactivation	6
1.3. Irradiation	7
1.4. Solvent extraction	8
2. Detoxification by Biological Methods	10
3. Detoxification by Chemical Methods	13
3.1. Oxidizing Agents	13
3.1.1. Chlorine	13
3.1.2. Hydrogen Peroxide	13
3.1.3. Ozone	14
3.1.4. Sodium Hypochlorite	15
3.1.5. Bisulfite	16
3.2. Acids and Bases	18
4. Decontamination of Aflatoxins by Ammoniation	21
4.1. Ammonia Treatment without Pressure	21
4.2. Ammonia Treatment with Pressure	29
4.3. Chemical Characteristic and Identification of the Major Reaction Product	30

	Page
Part II - Biological Effects	34
1. Biological Effects of Aflatoxins	34
2. Biological Effects of Ammoniated Aflatoxins	40
MATERIALS AND METHODS	45
1. Preparation of Contaminated Corn	45
2. Decontamination	45
2.1. Assembling of Ammoniation Unit.	45
2.2. Ammoniation Procedure	49
Preparation of Ammoniated Aflatoxins-Contaminated Corn	
3. Plan of Animal Feeding	50
3.1. Animals and their Management	53
3.2. Digestibility Trial	53
3.3. Collection of Samples	54
3.3.1. Blood Sampling	54
3.3.2. Excreta Sampling	54
4. Analytical Procedures	55
4.1. Determination of Aflatoxin	55
4.1.1. Preparation of Aflatoxin Standards Solution.	55
4.1.2. Extraction of Samples	56
4.1.3. Clean up of the Extract	57
4.1.4. Determination	57
4.2. Proximate Analysis	59
4.3. Serum Biochemical Parameters	59
4.3.1. Diagnostic Kits	59
4.3.2. Enzyme Activities	60

	Page
4.3.3. Total Proteins (TP)	60
4.3.4. Urea	61
4.3.5. Creatinine	61
4.3.6. Total Bilirubin	61
4.3.7. Albumin	61
4.3.8. Calculated Serum Globulin	62
4.3.9. Calculated Albumin (A): Globulin (G) Ratio	62
5. Statistical Analysis	62
RESULTS AND DISCUSSION	63
1. Study the Destruction of Aflatoxins in Corn Using ammonia	63
1.1. Effect of Different Corn bed thickness during Ammonia- tion	64
1.2. Effect of Different Concentrations of Ammonia	67
1.3. Effect of Different Temperatures During Ammoniation	69
1.4. Influence of Time of Ammoniation	71
1.5. Effect of Different Times of Agitation During Ammoniation	71
2. Toxicity of Ammoniated Aflatoxins-Contaminated Corn on Mice	75
2.1. Estimation of LD ₅₀ of Crude Aflatoxins B ₁ , B ₂ , G ₁ & G ₂	75
2.2. Clinical Symptoms and Mortalities	76
3. Effect of Aflatoxins-Contaminated Corn and Ammoniated Afla- toxins-Contaminated Corn on the Performance of Zaraiby Males Goats	77
3.1. Digestibility of Different Nutrients	81
3.2. Nitrogen Balance and Utilization of Nitrogen Intake	83
3.3. Liver Function	85

	Page
3.3.1. Transaminase (ALT) and (AST)	86
3.3.2. Serum Alkaline Phosphatase (SAP) Activity	91
3.3.3. Serum Protein Estimation	95
3.3.3.1. Serum Total Protein Content (g/dl)	95
3.3.3.2. Total Albumin Content (g/dl)	98
3.3.3.3. Total Globulins Content (g/dl)	102
3.3.3.4. Albumin/Globulin Ratio	105
3.4. Estimation of the Other Nitrogen Compounds	105
3.4.1. Estimation of Serum Urea	105
3.4.2. Estimation of Serum Creatinine	110
3.5. Test Based on Hepatic Secretions and Excretions	110
SUMMARY	117
REFERENCES	122
ARABIC SUMMARY	

LIST OF TABLES

Table	Page
1 The concentration of the toxins were calculated as (ppb) of aflatoxins-contaminated corn and ammoniated aflatoxins-contaminated corn	46
2 The chemical composition of the feeds (DM basis)	51
3 Chemical composition of total feeds as fed	52
4 Studied parameters	64
5 Mean values of destruction of aflatoxins at different conditions	65
6 Residual $\text{NH}_3\%$ at different temperature	69
7 Calculation of LD_{50} of the crude aflatoxins B_1 , B_2 , G_1 & G_2 in mice	75
8 Mean body weight (kg) and daily body weight changes (g) of male Zaraiby goats as affected by aflatoxins-contaminated corn and ammoniated aflatoxins-contaminated corn	78
9 Feed intake and nutrients digestibility coefficients of goats on different treatments during the different stages of experiment	80
10 Nitrogen intake, excretion and balance of goats during the different stages of experiment	84
11 Mean serum transaminases (AST & ALT) activity (IU/L) of male goat groups fed the control, ammoniated-aflatoxin or non ammoniated aflatoxin contaminated rations	87
12 Mean values of SAP for goat groups fed aflatoxin free, aflatoxin ammoniated and aflatoxin-contaminated ration	92

	Page
13 Mean values of total protein in serum (g/dl) of male goat groups fed aflatoxin free, aflatoxin ammoniated and aflatoxin-contaminated dities	96
14 Mean values of serum albumin in male goat groups fed aflatoxin free, aflatoxin ammoniated or aflatoxin-contaminated diets	99
15 Mean values of serum globulin (g/dl) of male goat groups fed aflatoxin free, aflatoxin ammoniated and aflatoxin-contaminated dities	103
16 Albumin/globulin ratio in serum of treated and untreated male goats with aflatoxins and ammoniated-aflatoxins	106
17 Mean values of urea in serum (mg/dl) of male goat groups fed aflatoxin free, aflatoxin ammoniated and aflatoxin-contaminated ration	108
18 Mean values of creatinine in serum (mg/dl) of male goat groups fed aflatoxin free, aflatoxin ammoniated and aflatoxin-contaminated diets	111
19 Mean values of total bilirubin in serum (g/dl) of male goat groups fed aflatoxin free, aflatoxin ammoniated and aflatoxin-contaminated diets	114

LIST OF FIGURES

Figure	Page
1 The chemical structure of aflatoxins	2
2 A proposed scheme for the ammoniation of aflatoxins B ₁ to produce aflatoxin D ₁ and mol.wt. 206 compound	32
3.a Schematic diagram of decontamination unit (ammoniation unit)	47
3.b Schematic set-up of adsorption column for ammoniation	48
4 Effect of different bed thickness on degradation of aflatoxins in artificially contaminated corn	66
5 Effect of different concentrations of ammonia on degradation of aflatoxins in artificially contaminated corn	68
6 Effect of different temperature on degradation of aflatoxins in artificially contaminated corn	70
7 Effect of different time of ammoniation on degradation of aflatoxins in artificially contaminated corn	72
8 Effect of different time of agitation on degradation of aflatoxins in artificially contaminated corn	73
9 Effect of feeding aflatoxins-contaminated corn or ammoniated aflatoxins-contaminated corn on serum alanine amino transferase of male goats	88
10 Effect of feeding aflatoxins-contaminated corn or ammoniated aflatoxins-contaminated corn on serum aspartate amino transferase of male goats	89

Figure	Page
11 Effect of feeding aflatoxins-contaminated corn or ammoniated aflatoxins-contaminated corn on serum alkaline phosphatase of male goats	93
12 Effect of feeding aflatoxins-contaminated corn or ammoniated aflatoxins-contaminated corn on serum total protein of male goats	97
13 Effect of feeding aflatoxins-contaminated corn or ammoniated aflatoxins-contaminated corn on serum albumin of male goats	100
14 Effect of feeding aflatoxins-contaminated corn or ammoniated aflatoxins-contaminated corn on serum globulin of male goats	104
15 Effect of feeding aflatoxins-contaminated corn or ammoniated aflatoxins-contaminated corn on serum albumin/globulin ratio of male goats	107
16 Effect of feeding aflatoxins-contaminated corn or ammoniated aflatoxins-contaminated corn on serum urea of male goats	109
17 Effect of feeding aflatoxins-contaminated corn or ammoniated aflatoxins-contaminated corn on serum creatinine of male goats	112
18 Effect of feeding aflatoxins-contaminated corn or ammoniated aflatoxins-contaminated corn on serum total bilirubin of male goats	115

INTRODUCTION

#####

INTRODUCTION

Aflatoxins, a group of naturally occurring toxins produced by the molds Aspergillus flavus and Aspergillus parasiticus, contaminate a variety of agricultural food and feed products (Jorgensen & Ralph, 1981). Corn, peanuts, and cottonseed are the major crops that are the most often contaminated by aflatoxins (Bagley, 1979).

Aflatoxins are potent hepatotoxins and also potent carcinogens (Bullerman, 1979) Fig. (1).

The effect of fungal infection on the decrease of the yield of different crops is a top priority problem and urgently needed to be considered. As a matter of fact the losses caused by fungal contamination is mainly due to the rejection of food with visible fungal growth and as well to its probable content of aflatoxins (Hopf, 1977).

In this concern, the Food and Agriculture Organization (FAO) estimates that 25% of the worlds food are affected by aflatoxins (Mannon and Johnson, 1985).

Some developing countries like Egypt is suffering from shortage of grains which is due to the extremely continuous increase in population and consumption with limited or even a successive decreasing area for cultivation. Accordingly many cereal crops are imported to cover the human and animal consumption. In this