# EFFECT OF AFLATOXIN B, TREATMENT ON PREGNANCY, NEWBORN, AND QUALITY AND QUANTITY OF MILK PRODUCED FROM MAMMALS

BY

#### MOSAAD ATTIA ABDEL-WAHHAB

B. Sc. Animal production 1981,M. Sc. Animal physiology 1989

A thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

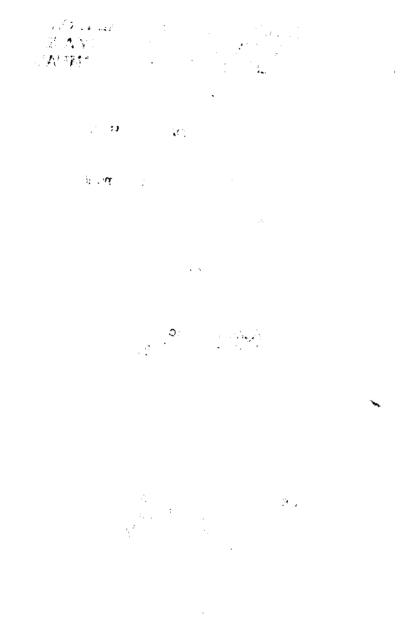
in

Agricultural Science (Animal physiology)

Department of Animal Production Faculty of Agriculture Ain Shams University

Cairo - Egypt

1996





# PREGNANCY, NEWBORN, AND QUALITY AND QUANTITY OF MILK PRODUCED FROM MAMMALS

BY

#### MOSAAD ATTIA ABDEL-WAHHAB

A thesis submitted in partial fulfillment of the requirements for the degree of

### DOCTOR OF PHILOSOPHY

in

Agricultural Science (Animal physiology)

Department of Animal Production
Faculty of Agriculture
Ain Shams University
Cairo - Egypt

1996



# **APPROVAL SHEET**

Effect of aflatoxin B<sub>1</sub> treatment on pregnancy, newborne, and quality and quantity of milk produced from mammals

By

Mosaad Attia Abdel-Wahhab

B.Sc. Animal production (1981)

M.Sc. Animal physiology (1989)

This thesis for Ph.D degree has been approved by:

Prof. Dr. E.A. Kotoby ( It Sayed Kett)

Professor of Animal Physiology, Ain-Shams University

Prof. Dr. A.A. Mohamed ( alle a . Tahampe

Professor of Animal Physiology, Al-Azhr University

Prof. Dr. S.O.Amin (S.O., Ari)

Professor of Animal Physiology, Ain-Shams University

Date of examination 11 / 1 /1996

# Effect of Aflatoxin B₁ treatment on pregnancy, newborn, and quality and quantity of milk produced from mammals

By

## Mosaad Attia Abdel-Wahhab B. Sc. Animal production 1981 M. Sc. Animal physiology 1989

Under the supervision of:

Dr. S. O. Amin,

Prof. of Animal Physiology, Ain Shams University, Cairo, Egypt.

Dr. Khayria Naguib,

Prof. of Mycotoxins, National Research Center, Cairo, Egypt.

Dr. K. Mayura,

Research Associate, Texas A&M University, College Station,

Texas, USA.



#### ABSTRACT

Mosaad Attia Abdel-Wahhab, Effect of Aflatoxin B<sub>1</sub> treatment on pregnancy, newborn, and quality and quantity of milk produced from mammals. Unpublished Ph.D of Science, University of Ain-Shams, Faculty of Agriculture, Department of Animal production, 1996.

In the rabbit study, 15 pregnant animals were divided into three groups, group 1 dosed orally with 0.15 mg and group 2 dosed orally with 0.1 mg AFB<sub>1</sub>/kg body weight during 12-30 days of gestation while group 3 was used as control. Treatment with AFB<sub>1</sub> significantly decreased GPT and GOT contents in the serum of pregnant rabbits. Histological examination of the ovaries collected at the end of the treatment period showed coagulative necrosis in the growing and mature follicles and, decreased number of Graffian and growing follicles with increased number of atretic follicles.

The effects of sorbents (HSCAS 1, HSCAS 2, and clinoptilolite) on AFB,-induced developmental toxicity were studied in 84 female rats. The sorbents were added to the diet at a level of 0.5% (w/w). Test animals were dosed orally with AFB, at 2.0 mg/kg body weight during gestation (days 6-13), and dams and fetuses were evaluated for maternal, developmental, and histological parameters on day 20 of gestation. AFB, al.one and clinoptilolite plus AFB, resulted in significant maternal and developmental toxicity. HSCAS1 and HSCAS 2 markedly diminished the maternal (i.e., mortality, reduction in body weight, ascites, hepatotoxicity, and decreased feed intake), and developmental (i.e., embryolethality and embryotoxicity) effects induced by AFB, when added to the diet at a concentration of only 0.5% w/w. Clinoptilolite was ineffective in preventing the maternal and developmental toxicity induced by AFB, in rats. Interestingly, clinoptilolite plus AFB, resulted in very severe maternal liver lesions (more than AFB, alone). None of the sorbents exhibited any toxicity either in maternal animals or developing embryos.

Thirty two adult male rats maintained on diets containing 0.5% (w/w) HSCAS 1, HSCAS 2 or clinoptilolite were dosed orally with 2.0 mg AFB<sub>1</sub>/kg body weight HSCAS 1 and HSCAS 2 markedly reduced the excretion of AFM<sub>1</sub> (major urinary metabolite) in the urine of rats compared to the clinoptilolite. The findings from this study confirm that HSCAS tightly bind AFB<sub>1</sub> and, the AFB<sub>1</sub>-HSCAS complex is not significantly dissociated *in vivo*. HPLC, CSID-M<sub>1</sub>, and ELISA were compared for

detection of AFM<sub>1</sub> in three kinds of milk. CSID-M<sub>1</sub> and HPLC methods were accurate and detected AFM<sub>1</sub> at concentrations as low as 0.125 ppb in milk samples. ELISA detected AFM<sub>1</sub> at a concentration of  $\geq$  0.25 ppb. This study demonstrates that the CSID-M<sub>1</sub> minicolumn method is rapid, accurate, cost-effective, and user friendly. This method is particularly useful for the field screening of milk and to predict exposure to AFB<sub>1</sub>. Key Words: Aflatoxin B<sub>1</sub>, Clay, rabbits, rats, pregnancy, newborn, milk aflatoxin M<sub>1</sub>.

### **ACKNOWLEDGMENTS**

My life is an eventful one, and invariably these events involve one or more individuals. These individuals contributed directly and indirectly to this degree and I would like to thank all of them. I would like to thank all the members of my committee for their comments, constructive criticisms on this work. I would like to thank Dr. Safaa O Amin, Dr. Khayria Naguib, Dr. Timothy Phillips, and Dr. Kittane Mayura for their interest, guidance and invaluable assistance that took me each day and made my life so much easier in many ways. I would like to say a special thank you. Drs. Wafaa E Abdel-Aal, Esam Tharwat, A B Sarr, and John F. Edwards, for providing me with a needed assistance.

I would like to acknowledge Dr. Hart Baily, Dr. Kent Washburn, Dr. Huang Zhao, Patrick Grant, Feng Zhao, Scott Mckenzie, Paul Herrera, and Maxene Dwyer, Margaret Caskey, and many others who supported me in one form or another and allowed me to achieve this goal. I especially want to thank all the staff members in the Mycotoxins Central Laboratory, National Research Center, and Department of Animal Production, Ain Shams University, for their enthusiastic assistance and good spirit of cooperation.

Finally I would like to extend a very special thank you to my mother, my wife, my sons Amir and Anas, my brothers and sisters for their good understanding, blessings, love and continuous help which made me easy to fulfill my dream of my life.

# **CONTENTS**

	Page
I: INTRODUCTION	1
II: REVIEW OF LITERATURE	4
1: Historical aspect	4
2: Chemical structure and properties of aflatoxins	6
3: Effects of Aflatoxins on Liver	12
3-1: Acute And Chronic Toxicity of Aflatoxins	12
3-1-1: Acute Exposure	13
3-1-2: Chronic Exposure	15
3-2: Mechanisms of Toxicity	16
3-3: Histopathological and Biochemical Observations	17
3-4: Enhancement and Inhibition of Toxicity	18
3-5: Role of Acute and Chronic Toxicity in Carcinogenic	ity 20
3-5-1: Increased Cell Proliferation	21
3-5-2: Cell Replication in Carcinogenesis	21
3-5-3: Cell Replication in Response to Aflatoxins	<b>2</b> 2
3-5-4: Implications of Aflatoxin Toxicity for Hepatic	
Carcinogenesis	24
4: Nonhepatic Disposition and Effects of Aflatoxin B <sub>1</sub>	25
4-1 Effects of Aflatoxin B <sub>1</sub> on the Respiratory System	25
4-2 Effects of AFB <sub>1</sub> on the Renal System	29
4-3 Effects of AFB <sub>1</sub> on the Gastrointestinal System	31
4-4 Effects of AFB <sub>1</sub> on the Nervous System	32
4-5: Effects of AFB <sub>1</sub> on the Reproductive System	34
4-6 Effects of AFB <sub>1</sub> on the Immune System	35
4-7 Other Effects of AFB <sub>1</sub>	39
5- Developmental Toxicities of Aflatoxins in Pregnant Anim	
6- Controlling and Prevention of Toxicity of Aflatoxins	41
6-1: Food and feed processing	41
6-2: Thermal Inactivation	42

6-3: Irradiation	43
6-4: Solvent Extraction and Mechanical Separation	44
6-5: Density Segregation	44
6-6: Adsorption from Solution	45
6-7: Biocontrol and Microbial Inactivation	45
6-7-1: Structural Degradation Following Chemical Treatme	nt 47
6-7-2: Ammoniation	47
6-7-3: Treatment with Bisulfite	49
6-7-4: Heterogeneous Catalytic Degradation	50
6-8: Reduction in Bioavailable Aflatoxin by Selective	
Chemisorption	50
7: Biotransformation and Metabolism of Aflatoxin B <sub>1</sub>	52
7-1: Biotransformation and Aflatoxin Toxicity	52
7-2: Detoxification Pathways	53
7-3: Biotransformation as a Determinant of Susceptibility	
to Aflatoxin Toxicity	53
7-4: Oxidation	55
7-5: hydroxylation	58
7-6: Odemethylation	59
7-7: AFB <sub>2a</sub> and AFG <sub>2a</sub> Formation	60
7-8: Enzymology of Aflatoxin Oxidations	61
7-9: Reduction	63
7-10: Conjugation	63
8: Factors Affecting Aflatoxin Biotransformation	64
8-1: Dietary Factors	64
8-2: Drug Treatments	66
9: Occurrence and Detection of Aflatoxin M <sub>1</sub>	67
9-1: Occurrence of aflatoxin M1	67
9-2: Detection methods of aflatoxin M <sub>1</sub> in milk	69
III: MATERIALS AND METHODS	81
1 : Evaluation of the toxicity of AFB <sub>1</sub> in New Zealand	
pregnant rabbits	81
1-1 : Chemicals	81
1-2 : Experimental animals	81