UNIVERSITI PUTRA MALAYSIA

STUDIES ON THE AETIOPATHOGENESIS AND PREVENTION OF *BRACHIARIA DECUMBENS* INTOXICATION IN SHEEP IN MALAYSIA

SHIZHEN ZHANG

FPV 2000 5

STUDIES ON THE AETIOPATHOGENESIS AND PREVENTION OF *BRACHIARIA DECUMBENS* INTOXICATION IN SHEEP IN MALAYSIA

By

SHIZHEN ZHANG

Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Veterinary Medicine Universiti Putra Malaysia

May 2000

With appreciation and respect,
this thesis is dedicated

To my grandfather,
who inspired me with confidence and ambitions.

To all my supervisors,
here and abroad,
who ensured it all worthwhile.

To my wife and my daughter,
who made this work endurable.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

STUDIES ON THE AETIOPATHOGENESIS AND PREVENTION OF *BRACHIARIA DECUMBENS* INTOXICATION IN SHEEP IN MALAYSIA

By

SHIZHEN ZHANG

May 2000

Chairman: Dr. Noordin Mohamed Mustapha, Ph.D.

Faculty: Veterinary Medicine

The pathogenesis of *Brachiaria decumbens* intoxication has not been well explained leading to continuing outbreaks and ineffective treatment and control. Studies conducted in this thesis were aimed at elucidating the pathogenesis which in turn will lead to a better understanding of the disease and provide pragmatic strategies in treating and preventing *B. decumbens* intoxication.

Experiments were conducted to obtain a baseline value of mineral and phytate levels in *B. decumbens*, the effect of feeding *B. decumbens* to sheep, the role of copper (Cu) in *B. decumbens* intoxication, the effectiveness of zinc (Zn) and its role in the prevention of *B. decumbens* intoxication and the isolation, characterisation and toxicity testing of the compound in *B. decumbens*.

Samples of *B. decumbens* collected from five different farms representing Peninsular Malaysia were air-dried, milled and analysed for the concentration of selected minerals and phytate. In three other separate experiments, sheep were fed *B. decumbens* either alone, with Cu or Zn or a combination of Cu and Zn. Copper and Zn were given orally in gelatin capsules on five days of the week at a dosage of 15 mg of Cu as $CuSO_4 5H_2O$ and 25 mg of ZnO/kg body weight respectively. Blood and pertinent tissues were collected at fortnightly intervals and necropsy respectively. The liver function enzymes, pertinent indicators reflecting antioxidant defense and lipid peroxidation and selected minerals were

monitored, and pathologic studies both at cellular and subcellular levels were also conducted.

A non-critical variation of concentration of Cu, Fe and Zn and low phytate was found in all samples. Clinical signs of photosensitisation, jaundice and submandibular oedema and lesions of hepatocytic necrosis and renal damage are seen in sheep *fed B. decumbens* either alone or in combination with Cu and/or Zn. In all *B. decumbens* fed sheep, there was an impairment of the antioxidant defense and involvement of lipid peroxidation. The concentration of Cu, Fe and Zn in the grass *per se* is not involved in this intoxication. However, excess Cu and Zn further exacerbated the progression of the intoxication. Thus, Zn is ineffective in preventing the development of *B. decumbens* intoxication. Supplementation of Cu-rich by-products at any stages of *B. decumbens* feeding is not recommended.

Studies on the isolation and characterisation of the toxic compound yielded a diosgenin. Toxicity test of diosgenin from *B. decumbens* in mice indicated a LD_{50} of 410.5 mg/kg.

Based on the results from studies conducted, the pathogenesis of this intoxication is postulated as follows: after uptake by hepatocytes, sapogenin and its metabolites undergoes biotransformation, which is catalysed by mixed function oxidases system, yielding intermediate free radicals; hepatocytes damage at both cellular and subcellular levels due to membrane lipid peroxidation leading to interference in the transport and excretion of endogenous metabolites which in turn lead to jaundice and photosensitisation.

Abstrak tesis yang dikemukakan pada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KAJIAN MENGENAI ETIOPATOGENESIS DAN PENCEGAHAN KERACUNAN *BRACHIARIA DECUMBENS* PADA BEBIRI DI MALAYSIA

Oleh:

SHIZHEN ZHANG

Mei 2000

Pengerusi: Dr. Noordin Mohamed Mustapha, Ph.D.

Fakulti: Perubatan Veterinar

Akibat kekurangan maklumat, keracunan *B. decumbens* masih berlaku tanpa ujud rawatan dan kawalan yang berkesan. Kajian yang dijalankan dalam tesis ini bertujuan untuk menerangkan patogenesis yang akan memudahkan perancanagan strategi rawatan dan kawalan yang pragmatik.

Ujikaji telah di jalankan untuk membentuk asas kandungan mineral dan fitat dalam *B. decumbens,* kesan meragut rumput ini, peranan kuprum (Cu) dan keberkesanan zink (Zn) dalam keracunan *B. decumbens* dan pengasingan, pencirian serta ujian ketoksikan sebatian dalam *B. decumbens.*

Sampel rumput yang di perolehi daripada lima buah ladang ternakan di Semenanjung Malaysia dikeringkan, dikisar dan dianalisis untuk kandungan mineral terpilih dan fitat. Dalam tiga lagi ujikaji yang berasingan, bebiri diberi makan *B. decumbens* samada secara langsung atau digabung dengan Cu atau Zn atau keduanya. Dos bagi Cu dan Zn adalah masing-masing 15 mg Cu sebagai $CuSO_4 \cdot 5H_2O$ dan 25 mg/kg Zn sebagai ZnO 25mg secara oral pada lima hari setiap minggu. Darah dan tisu berkaitan masing-masing di ambil setiap dua minggu sekali atau ketika nekropsi. Enzim fungsi hepar dan petunjuk pertahanan anti-pengoksid dan pengoksidaan lipid serta kepekatan mineral terpilih dan kajian patologi pada peringkat sel dan subsel turut dikaji.

Ketidakseragaman yang bukan kritikal secara taburan kawasan di perolehi dalam kepekatan Cu, Zn dan Fe serta kandungan fitat yang rendah dalam *B. decumbens*. Petanda seperti fotopekaan, jaundis, oedema submandibel dan lesi nekrosis hepatosit dan kerosakan ginjal dilihat pada bebiri yang memakan *B. decumbens* secara bersahaja ataupun digabung dengan Cu atau/dan Zn. Pada semua bebiri yang memakan *B. decumbens*, terdapat gangguan sistem pertahanan anti-pengoksid dan penglibatan pengoksidaan lipid. Kepekatan Cu, Fe dan Zn *per se* dalam rumput tidak terlibat dalam ketoksikan ini. Bagaimanapun, lebihan Cu dan Zn boleh memanjurkan kesan ketoksikan. Dengan itu, Zn didapati sebagai tidak berkesan dalam mencegah pembentukan ketoksikan *B. decumbens.* Penambahan bahan sampingan kaya-Cu pada mana-mana peringkat pemakanan *B. decumbens* adalah tidak di galakan.

Kajian mengenai pengasingan dan pencirian sebatian toksik menemukan diosgenin. Ujian ketoksikan diosgenin pada mencit memberikan LD_{50} sebanyak 410.5 mg/kg.

Berdasarkan keputusan kajian yang di jalankan, patogenesis ketoksikan ini dipostulatkan seperti berikut: selepas diambil oleh hepatosit, sapogenin dan metabolitnya mengalami biopenjelmaan, yang mana dikatalis oleh sistem oksidase fungsi tercampur, menghasilkan radikal bebas; hepatosit menyebabkan kerosakan di peringkat sel dan subsel kerana pengoksidaan selaput lipid mengakibatkan gangguan dalam pengangkutan dan perkumuhan yang seterusnya menyebabkan jaundis dan fotopekaan.

ACKNOWLEDGEMENTS

At the time of completing this thesis, I would like to express my profound appreciation and gratitude to everyone who has provided guidance and assistance, without which the completion of this thesis would have been impossible.

I am deeply indebted to my supervisors Dr. Noordin Mohamed Mustapha, Prof. Dato' Sheikh-Omar Abdul Rahman, and Associate Prof. Dr. Md Jelas Haron for their constant encouragement, unfailing help and tolerant supervision to my endeavours. Their suggestions, criticisms and provoking discussions have been most valuable.

I wish to express my gratitude to Dr. Mohamed Azmi Lila for his kindness in allowing me to pursue pertinent works in his laboratory. I am grateful for the enthusiatic and technical assistance of Dr. Gwendoline Ee Cheng Lian and Mr. Sugeng Riyanto. I am also indebted to Mr. R. Kumar for managing animals. My sincere thanks go to Mr. Noraziman Sulaiman and staffs of the post mortem laboratory, Mr. Ho Oi Kuan & Miss Azilah Abd Jalil from the EM unit of the Institute of Bioscience for their technical assistance and convenience. My gratitude is also extended to Dr. Md Aspollah Sukari for granting permission to use his laboratory facilities, and Mr. Kamal Margova of Department of chemistry, Mr. Pauzi Zakaria of Department of biochemistry for provision of facilities and the appropriate techniques.

I wish to thank all my colleagues especially the post-graduate students Dr. M. H. Al-Haddawi, H. Hazilawati and K. Y. Lai for their understanding and cooperation.

The study was undertaken while I was in receipt of the Graduate Assistant Scholarship of Universiti Putra Malaysia and study leave from Institute of Animal Science & Veterinary Pharmaceutics, CAAS, China. The project was supported under the IRPA 51183 grant by the Ministry of Science, Technology and Environment, Malaysia.

Finally, I would like to express my deepest gratitude and appreciation to my parents, my wife Fenglan Hao and my daughter Yiying Zhang for their unfailing support and encouragement, and for accepting my long time absence from the family that my commitment to this thesis necessitated.

I certify that an Examination Committee met on May 29, 2000 to conduct the final examination of Shizhen Zhang on his Doctor of Philosophy thesis entitled "Studies on the Aetiopathogenesis and Prevention of *Brachiaria decumbens* Intoxication in Sheep in Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Jasni Sabri, Ph.D
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Noordin Mohamed Mustapha, Ph.D
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Sheikh Omar Abdul Rahman, MRCVS
Dato' Professor/Dean
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Md. Jelas Haron, Ph.D
Associate Professor
Department of Chemistry,
Faculty of Sciences & Environmental Studies,
Universiti Putra Malaysia
(Member)

MOHD GHAZALI MOHAYIDIN, Ph.D,
Professor/Deputy Dean of Graduate School,
Universiti Putra Malaysia.

Date: 06 JUN 2000

This thesis was submitted to the Senate of Universiti Putra Malaysia and was accepted as fulfilment of the requirements for the degree of Doctor of Philosophy.

KAMIS AWANG, Ph. D.
Associate Professor,
Dean of Graduate School,
Universiti Putra Malaysia

Date: 13 JUL 2000

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations that have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

SHIZHEN ZHANG

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AAS	atomic absorption spectrophotometer
ALT	alanine aminotransferase
AST	aspartate aminortransferase
AP	alkaline phosphatase
Bd	group feed with *Brachiaria decumbens*
BSP	bromosulphopthalein
Ca	calcium
CCl_4	carbon tetrachloride
cm	centimetre
CP	crude protein
Cp	caeruloplasmin
Cu	copper
$CuSO_4$	copper sulphate
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAS	department of animal science
DM	dry matter
DW	dry weight
E-SOD	superoxide dismutase in erythrocytes
FLCC	flash-column chromatography
Fe	iron
g	grams
g.Pr	per gram of protein
GC-MS	gas chromatography-mass spectroscopy
GLDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GSH-Px	glutathione peroxidase
H_2O_2	hydrogen peroxide
Hb	haemoglobin
HPS	hepatogenous photosensitisation syndrome
H-SOD	superoxide dismutase in liver homogenate
H&E	haematoxylin and eosin staining
ICP	Inductively coupled plasma technique
IR	infrared spectroscopy
K	potassium
kg	kilograms
l	litre
LD_{50}	median lethal dose
MDA	malondialdehyde
MFOs	mixed function oxidases system
Mg	magnesium
ml	millitre
mg	milligrams
mg.Hb	per milligram haemoglobin
mg.Pr	per milligram of protein
mmol	millimolar

Mo	molybdenum
MT	metallothionein
Na	sodium
nmol	nanomolar
NMR	nuclear magnetic resonance spectroscopy
O_2^-	superoxide anion
OH	hydroxyl radical
P	phosphorus
PA	pyrrolizidine alkaloids
PAS	Periodic Acid-Schiff staining
PAS+D	PAS positive after diastase digestion
PB	phenobarbitone
RBC	red blood cell
PKC	palm kernel cake
rER	rough endoplasmic reticulum
S	sulphur
SD	standard deviation
SDH	sorbitol dehydrogenase
sER	smooth endoplasmic reticulum
SOD	superoxide dismutase
SPDM	sporidesmin
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reactive substances
TLC	thin-layer chromatography
TM	thiomolybdate
UP	upper portion
UPM	Universiti Putra Malaysia
WP	whole grass
ww	wet weight
Zn	zinc
ZnO	zinc oxide
μ	micro
%	percentage