



UNIVERSITI PUTRA MALAYSIA

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

SHIZHEN ZHANG

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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

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

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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 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 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 LD₅₀ 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**

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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 perancangan 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 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 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.

Ketidakteraturan 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, lebih 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 LD₅₀ 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.

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LIST OF ABBREVIATIONS

AAS	atomic absorption spectrophotometer
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AP	alkaline phosphatase
Bd	group feed with <i>Brachiaria decumbens</i>
BSP	bromosulphophthalein
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 ₂ O 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	millilitre
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