UNIVERSITI PUTRA MALAYSIA

EFFECTS OF HYDROGEN CYANIDE IN CASSAVA (MANIHOT ESCULENTA CRANTZ) FORAGE ON RUMEN METABOLISM AND BODY TISSUES OF SHEEP AND GOATS

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MOHD ROSLY SHAARI, D.V.M

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, In Fulfilment of the Requirement for the Degree of Master of Science

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DEDICATION

“Dedicated especially to my wife, Dr. Hazilawati Hamzah, my father and mother Shaari Ahmad and Mardiah Omar, my father and mother-in-law Hamzah Yusof and Kelesom Silong and to my brothers whose sacrifice and support has enabled me to complete this study successfully”.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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Cassava leaves are good source of protein and have a potential to substitute grain concentrate in livestock feed. However, a major constraint in using cassava forage as animal feed is the presence of hydrogen cyanide (HCN). The objectives of the study were to determine the effects of hydrogen cyanide in cassava forage on rumen metabolism and body tissues and the tolerance level of sheep and goats. The first experiment was conducted to determine the total cyanogens in two varieties of local cassava forages, Black Twig (BT) and MARDI 92 (MM 92); and to determine the practical processing techniques in reducing the cyanide content to a safe level for animal feeding. Fresh BT contained high level of cyanide (1273 ppm) as compared to MM 92 (850 ppm). Slow drying as in under shade and sun drying, were found to be more effective in reducing cyanide content than rapid drying in forced draught oven. The second experiment was conducted to determine the effects of HCN and its tolerance level in sheep and goats. Nine male Dorset crossbred sheep and nine

Kambing Katjang goats of similar sex, weighing an average of 21.7 ± 1.1 kg each, were used. Three animals of each species were assigned to each of the three treatment groups namely control, HCN $_{4mg}$ (4 mg HCN/ kg body weight) and HCN $_{7mg}$ (7 mg HCN/ kg body weight) in a Complete Randomised Design (CRD) experiment. The animals except in control group were fed in addition to hay, pelleted MM 92 cassava leaves containing 311.7 ppm HCN (DM basis), to the required HCN levels. The serum and urine thiocyanate (SCN) in sheep and goats of both HCN treatment groups were significantly ($p<0.05$) higher as compared to the control group. The average detoxification rate of cyanide to thiocyanate was 3.6 and 3.0 ppm/hr for HCN $_{7mg}$ group and 2.1 and 2.3 ppm/hr for HCN $_{4mg}$ group in sheep and goats, respectively. Thiocyanate was shown to have a goitrogenic action that resulted in significant ($p<0.05$) reduction in thyroxine (T_4) level (1.5 μg/dl), increased in thyroid stimulating hormone (TSH) level (1.2 μIU/mL) and development of hyperplastic goitre in goats, while sheep were not severely affected. The serum aspartate aminotransferase (AST) activities were significantly ($p<0.05$) increased in both species, which were related with the presence of periportal necrosis of the liver. Mild nephrosis of the kidney was also observed. The presence of cyanide in the rumen had no significant effects ($p>0.05$) on the volatile fatty acids (VFA) production in the rumen. However, at HCN $_{7mg}$ treatment rumen microbial productions particularly in goats decreased by 16.7% ($p<0.05$). It could concluded that at the MLD of 7 mg HCN/kg body weight, there were significant toxicity effects to sheep and goats, while at the MLD of 4 mg HCN/kg body weight the adverse effects were restricted to the serum and urinary thiocyanate concentrations only. Based on the thyroid hormones and histology, sheep are more tolerance to cyanide as compared to goats.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Mater Sains

KESAN HIDROGEN SIANIDA DALAM FURAJ UBI KAYU (*MANIHOT ESCULENTA* CRANTZ) KE ATAS METABOLISMA RUMEN DAN TISU BADAN BEBIRI DAN KAMBING

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Daun ubi kayu adalah sumber protin yang baik dan berpotensi untuk menggantikan konsentrat bijirin dalam makanan ternakan. Halangan dalam penggunaannya sebagai makanan haiwan adalah kehadiran hidrogen sianida (HCN). Tujuan ujikaji ini dijalankan adalah untuk menentukan kesan hidrogen sianida dalam furaj ubi kayu ke atas metabolisma rumen dan tisu badan dan toleransinya pada bebiri dan kambing. Eksperimen pertama telah dijalankan untuk menentukan jumlah sianida di dalam dua jenis furaj ubi kayu tempatan, Black Twig (BT) dan MARDI 92 (MM 92); dan menentukan teknik pemprosesan yang praktikal untuk mengurangkan kandungan sianida ke paras yang selamat untuk makanan haiwan. BT segar mengandungi paras sianida yang tinggi (1273 ppm) berbanding MM 92 (850 ppm). Pengeringan perlahan seperti di bawah teduhan dan matahari, didapati lebih berkesan dalam mengurangkan kandungan sianida berbanding pengeringan cepat di dalam oven. Eksperimen kedua telah dijalankan untuk menentukan kesan-kesan sianida dan

tahap toleransinya pada bebiri dan kambing. Sembilan ekor bebiri jantan Kacukan Dorset dan sembilan ekor Kambing Katjang jantan, dengan purata berat 21.7 ± 1.1 kg seekor, telah dipilih untuk kajian ini. Tiga ekor haiwan dari setiap spesis telah dibahagikan kepada tiga kumpulan rawatan iaitu kawalan, HCN_{4mg} (4 mg HCN/kg berat badan) dan HCN_{7mg} (7 mg HCN/kg berat badan) dalam ekperimen dengan Rekabentuk Rawakan Lengkap (CRD). Ternakan kecuali dalam kumpulan kawalan diberi makan pelet daun ubi kayu MM 92 mengandungi 311.7 ppm HCN (bahan kering), ke paras-paras HCN yang dikehendaki. Tiosianida (SCN) serum dan urin bagi bebiri dan kambing dalam kedua-dua kumpulan rawatan adalah lebih tinggi ($p<0.05$) berbanding kawalan. Purata kadar detoksifikasi sianida ke tiosianida masing-masing adalah 3.6 dan 3.0 ppm/jam untuk kumpulan HCN_{7mg}, dan 2.1 dan 2.3 ppm/jam untuk kumpulan HCN_{4mg}, pada bebiri dan kambing. Tiosianida didapati mempunyai tindakan kebegukan yang menyebabkan penurunan ($p<0.05$) paras T_4 (1.5 µg/dl), peningkatan paras TSH (1.2 µIU/mL) dan pembentukan beguk hiperplastik pada kambing, manakala bebiri pula tidak teruk terjejas. Aktiviti AST serum meningkat ($p<0.05$) dalam kedua-dua spesis, berkaitan dengan kehadiran nekrosis periportal hati. Neprosis ringan pada buah pinggang juga diperhatikan. Kehadiran sianida dalam rumen tidak menyebabkan kesan ($p>0.05$) pada produksi asid lemak meruap (VFA) rumen. Pada rawatan HCN_{7mg} produksi mikrob rumen kedua-dua spesis menurun sebanyak 16.7% ($p<0.05$). Kesimpulannya, pada MLD 7 mg HCN/kg berat badan, sianida menyebabkan kesan-kesan keracunan signifikan pada kambing dan bebiri, sementara itu pada MLD 4 mg HCN/kg berat badan kesan-kesan bahaya sianida hanya terhad pada tiosianida serum dan urin sahaja. Berdasarkan hormon tiroid dan histologi, bebiri adalah lebih toleran terhadap sianida berbanding kambing.

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LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS

ANOVA	analysis of variance
AOAC	Association Official Agricultural Chemists
AST	aspartate aminotransferase
BT	black twig
CK	creatinine kinase
CN	cyanide
CNO	cyanate
CO_2	carbon dioxide
CRD	complete randomised design
DIT	diiodotyrosine
DM	dry matter
FAO	Food and Agriculture Organisation
HCN	hydrogen cyanide
HCN_{4mg}	4 mg HCN/kg body weight group
HCN_{7mg}	7 mg HCN/kg body weight group
H & E	haematoxylin & eosin
HNL	hydroxynitrilyse
HPLC	high performance liquid chromatography
IRMA	immunoradiometric assay
MARDI	Malaysia Agriculture and Research Development
MIT	monoiodotyrosine
MLD	minimum lethal dose
MN	microbial nitrogen
NFE	nitrogen free extract
NPN	non protein nitrogen
PD	purine derivatives
PPM	part per million
RIA	radioimmuno assay

RT	room temperature
SCN	thiocyanate
S-SCN	serum thiocyanate
TSH	thyroid stimulating hormone
T_4	thyroxine
T_3	triiodotyronine
U-SCN	urinary thiocyanate
VFA	volatile fatty acids
WHO	World Health Organisation

CHAPTER I

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) also known as tapioca or manioc, is one of the major tuber crops grown in more than 80 countries in the humid tropics. It is a high-energy food obtained with low nutrient inputs. It is either a main or a secondary staple food (Cock, 1985) for the people in the tropics. Besides its important role in human nutrition, cassava has also been used as a feedstuff for livestock (Maner and Gomez, 1973).

The cassava leaves and shoots are rich nutritionally in protein and vitamins (Lancaster and Brooks, 1983). Research has shown that cassava leaves have protein content ranging from 16.7 to 39.9% with almost 85% of the crude protein fraction present as a true protein (Ravindran, 1991). Wanapat *et al*. (1997) showed that cassava hay fed either as a whole ration or as a supplement in crop-residue based diets is a good feed for ruminant. Recently, Che (2001) reported that the ruminal degradability of cassava fodder was as high as that of grain concentrates such as maize and soy bean meal at the same outflow rates. Therefore, cassava fodder can be used as protein supplement to substitute grain concentrates up to 50% in ruminant diet.

However, its widespread use as animal feed is limited by the presence of hydrogen cyanide (HCN), which has to be properly reduced to a safe level. It is well established that the toxicity of cassava is due to the release of free cyanide from cyanogenic glycosides, linamarin and lotaustralin. Level of cyanide in plant material in excess of 200 ppm is potentially dangerous to livestock (Buck and Osweiler, 1976). Frequent intake of cassava would lead to the ingestion of small quantities of cyanide. Reliable information on the toxicity to sheep and goats caused by the cumulative effects of ingested cyanide is very limited (Nambisan, 1994). In addition, information on the comparative tolerance to the cyanogenic compound in ruminant livestock such as sheep and goats has not been reported.

Experiments reported in this thesis were conducted to substantiate information on the utilisation of cassava forages as a protein supplement in small ruminants. Therefore, the objectives of the experiments were:

i). to determine the cyanide content in two varieties (MM 92 and BT) of cassava forages

ii). to evaluate the effect of practical processing technique to reduce the cyanide content

iii). to compare differences between sheep and goats in the level of tolerance to cyanide ingestion

iv). to assess the short term effects of consuming hydrogen cyanide in cassava on the health and performance of sheep and goats.

CHAPTER II

LITERATURE REVIEW

The Cassava Plant

Cassava belongs to the family Euphorbiaceae, which includes rubber (*Havea brasilensis*) and castor bean (*Ricinus communis*). It is one of the most widely cultivated tuber crops serving as the major staple food of more than 300 million people in the tropics (Cock, 1982).

Botanical Description

Cassava is a perennial woody shrub producing enlarged tuberous roots and variously branched stems. The height, spread and other characteristics vary among cultivars. Usually cassava attains a height varying from 1 to 5 m. The shrubs of cassava may be multi branched or unbranched (Cock, 1985). In the branching types of cassava, branching may occur at the base, midway, or at the top of the stem. The number of branches varies from 1 to 6 depending on the variety. The stem of the cassava plant is tall, thin and straight and has a number of nodes. The colour of the stem usually varies from red-brown to grey. The leaves of cassava are palmate possessing 3 to 11 lobes. The primary leaves of seedlings are unlobed whereas secondary leaves are 3-lobed (Balagopalan *et al*., 1988).

Commercially, cassava is grown by planting a cutting taken from the woody part of the stem. The growth cycle of a typical cassava crop is close to 1 year. The roots start bulking about 3 months after planting and continue to increase in weight until 9 to 15 months after planting when the crop is usually harvested.

Origin, Spread and Adaptation

Cassava is native of tropical America, until recently Brazil was considered to be the place of origin for cassava. In Africa, cassava was introduced in the 16^{th} century. The Portuguese introduced cassava to India in 1840. In Java it was introduced around 1810. The first official record of cassava introduction into Malaysia was in 1886, through the Singapore Botanic Gardens (Cock, 1985); however cassava was widely grown in Malaysia before that, probably after introduction from India or Indonesia.

The cassava crop is cultivated under different climatic and soils conditions, for example, in the high rainfall areas of the Andes to the acid infertile soils of the Savannas. Although different varieties and agronomic practices are used, the fact that the species can be grown under these varied conditions demonstrates its broad adaptability.

Climate

Cassava is grown almost exclusively in the hotter lowland tropics and is never grown as crop further from the equator then 30°N or 30°S (Cock, 1985). It is grown at altitudes up to 2000 m, but can be grown profitably at lower altitudes in areas ranging from humid (more than 2000 mm annual rainfall) to semiarid (500 to 750 mm) conditions. The optimum temperature range for the growth of cassava is between 18 to 30°C.

It is a drought tolerant crop and can be grown in areas where there are occasionally prolonged spans of drought. During a drought period the plant regulates transpiration rate and enters a dormant state, shedding older leaves and putting forth fewer new leaves. With the onset of rains, reserves carbohydrates from the roots and stem are utilised and the plant become active again. Cassava is basically adapted to a tropical environment and yield well under full sunlight when the soil moisture is not limiting.

Soils

Cassava can tolerate a wide range of soil conditions (Balagopalan *et al.*, 1988). It grows well in soils of low fertility status and produces a satisfactory yield. A well-drained loamy or sandy loam with sufficient organic matter is the best soil for the cultivation of cassava. Proper tuber formation takes place only in those soils that are loose and friable. On heavy clay and rocky soils, the yield will be poor due to restricted tuber development. Poor drainage and water logging are also unfavourable for growing cassava. The optimum pH of the soil for cultivation is from 6.0 to 7.5, but it is grown under the wide range of pH in many parts of the world. In Malaysia,