ISSN: 1511-3701
Pertanika J. Trop. Agric. Sci. **33 (1)**: 127 - 133 (2010)

Tissues Thiocyanate (SCN) Concentration and Liver Pathology of Sheep and Goats Fed on Cassava Forages

S.M. Rosly[1*], J.B. Liang[2], M.M. Nordin[3], N. Somchit[4] and Z.A. Jelan[5]
[1]Strategic Livestock Research Centre, MARDI Headquarters, Serdang, Malaysia
[2]Institute of Bioscience,
[3]Department of Veterinary Pathology and Microbiology,
Faculty of Veterinary Medicine,
[4]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences,
[5]Department of Animal Science, Faculty of Agriculture,
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[*]E-mail: rosly@mardi.gov.my

ABSTRACT

Cassava leaves are good sources of protein which have a potential to substitute grain concentrate in livestock feed. However, a major constraint in using cassava fodder as animal feed is the presence of hydrogen cyanide (HCN). A study was conducted to compare the cumulative effects of thiocyanate (a product from the detoxification of hydrogen cyanide) at 4 mg and 7 mg HCN/ kg body weight on sheep and goats. Thiocyanate was sourced from the detoxification of hydrogen cyanide in cassava. The tissue thiocyanate concentrations were found to be significantly ($p<0.05$) higher in liver (2.29 µg/mL/g tissue) of goats as compared to that of sheep. Meanwhile, histological examination of the liver revealed the presence of periportal necrosis. In spite of detoxification process of hydrogen cyanide to thiocyanate, it could be concluded that at 7 mg HCN/kg body weight, considerable amount of thiocyanate was retained in the body and accumulated in the liver.

Keywords: Cassava, goats, hydrogen cyanide, liver, periportal necrosis, sheep, thiocyanate

INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is one of the major tuber crops grown in more than 80 countries in the humid tropics. It is also a staple food for at least 500 million people in the tropics (Cock, 1985). Besides its important role in human diets, cassava has also been used as a feedstuff for livestock (Maner and Gomez, 1973). Research on the use of cassava, as an animal feed, has been carried out for almost a century. These studies clearly show the importance of cassava in animal nutrition. Cassava fodder has been proven to be a potential fodder source for ruminant feeding as it contains high contents of crude protein (CP), minerals, and vitamins. Moreover, the leaves of cassava have a CP content ranging from 16.7 to 39.9% dry matter (DM), with almost 85% of the CP fraction present as a true protein (Ravindran, 1991). Supplementing low quality feeds like rice straw or grass with cassava foliage has resulted in increased CP intake, digestion of fibre in the rumen (Khang and Wiktorsson, 2004), increased feed intakes, digestibility of diets and improved live weight gains (Dung *et al*., 2005). Wanapat *et al*. (1997) showed that cassava hay, fed either as

Received: 16 November 2009
Accepted: 11 January 2010
[*]Corresponding Author

a whole ration or as a supplement in crop-residue based diets, is a good feed for ruminant. 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 a protein supplement to substitute grain concentrates up to 50% in the ruminant diet. However, its widespread use as animal feed is limited by the presence of cyanide, which may affect both health and performance of animals.

It is well established that the toxicity of cassava is due to the release of cyanide from cyanogenic glucosides, linamarin, and lotaustralin. Fresh cassava foliage contains high levels of cyanogenic glucosides, which produce cyanide (HCN) toxin. The cyanogenic glucosides are a group of nitrile-containing plant secondary compounds which yield cyanide following their enzymatic breakdown. All cassava tissues, with the exception of its seeds, contain cyanogenic glucosides linamarin (> 90 % total cyanogen) and lotaustralin (< 10% total cyanogen) (McMahon *et al*., 1995). Meanwhile, the leaves were found to contain the highest cyanogenic glucoside level (5.0 g linamarin/kg fresh weight), whereas roots have approximately 20-fold lower linamarin levels. Cyanogenesis is initiated in cassava when the plant tissue is damaged. Ingestion of cassava can trigger several toxic manifestations due to the release of HCN from cassava cyanogenic glucosides. The incidence of acute poisoning from consumption of cassava is relatively rare since the amount ingested is often low. However, intake of cassava in the long run can lead to toxic conditions as the animal is exposed to the sub-lethal doses of cyanide for a prolonged period. The toxicity of cassava is due to the release of HCN in vivo, which is a potent cytotoxin, exerting a wide range of biological effects.

Chronic cyanide intoxication, due to consumption of sub-lethal doses of HCN, has been reported mainly in pigs and rats (Tewe and Iyayi, 1992) and dogs (Kamalu and Agharanya, 1991). Meanwhile, increased level of thiocyanate in blood was observed in gilt fed on fresh cassava diets (Tewe and Maner, 1981). Hill (1977) reported that with doses of 50 mg/kg body weight linamarin, mortality was produced in experimental rats. Moreover, pathophysiological changes have also been observed in dogs fed with "garri", i.e. a fermented cassava product. Feeding of garri caused a significant elevation of serum alanine aminotransferase (ALT) (Kamalu, 1993) and a reduction in thyroid hormone (Kamalu and Agharanya, 1991). It is important to note that a level of HCN in plant material in excess of 200 ppm is potentially dangerous to livestock. Coop and Blakely (1950) stated that the minimum lethal dose (MLD) for sheep is 2.4 mg/kg HCN body weight and the lethal dose has been estimated to be 4 to 5 mg/kg HCN body weight. In the latter case, the MLD for sheep is in an order of 7 mg/kg HCN body weight for normal eating rates. Ruminants are more susceptible to poisoning by cyanogenic plants than the non-ruminants, while sheep are said to be less susceptible than cattle if actual doses are considered. Nevertheless, reliable information on the toxicity to sheep and goats, caused by the cumulative effects of ingested cyanide, is still 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. Therefore, the objective of the present study was to assess the short-term effects of feeding different levels (4 and 7 mg HCN/kg body weight) of HCN in cassava forages on sheep and goats, with the focus on thiocyanate accumulation in selected tissues and liver morphology.

MATERIALS AND METHODS

Animals Management

Nine male sheep (Dorsett Malin crossbred) and nine male of Kambing Katjang goats, aged between 10-12 months old and with an average body weight of 21.7 ± 1.07 kg, were selected in this study. Prior to the experiment, the animals were treated for ecto- and endoparasites and they were also trained to adapt to cassava leaves as a part of their diets. After two weeks

of adaptation period, the sheep and goats were allocated into their respective treatment groups. All the animals were then kept in metabolic crate with free access to drinking water during the experiment.

Preparation of the Feed Samples

Cassava fodder of MM 92 variety was freshly harvested at about 8 weeks of age from the experimental plots at Universiti Putra Malaysia, Serdang, Malaysia. They were chopped, oven dried at 70 °C for 8 hours (to retain as much cyanide as possible in the dry fodder), and pelleted for animal feeding experiment. Sub-samples of the pelleted cassava leaves were grounded up through a 2 mm screen sieve and tested for cyanide content. Hay pellets were obtained from Strategic Livestock Research Centre, MARDI, Serdang.

Feeds and Feeding

During the adaptation period, the animals were fed with hay and concentrate. Meanwhile, the animals in the control group were fed with only hay during the experiment. Those in the other two treatment groups were fed, in addition to hay, with pelleted cassava leaves at 3% DM of their body weight to the required HCN levels. The diets for the three groups were 100% hay (control), 45% cassava + 55% hay (4 mg/kg HCN), and 75% cassava + 25% hay (7 mg/kg HCN). The daily feed was given once at 9.00 a.m.

Experimental Design

Three animals of each species were randomly assigned to each treatment group, namely control (no HCN), low level HCN (4 mg HCN/kg body weight), and high level HCN (7 mg HCN/kg body weight) in a Complete Randomised Design (CRD). The experiment took 3 weeks to be completed. The animals were fed with cassava leaves containing 311.7 ppm HCN (DM basis), with each animal received zero (control), 87 mg/day (low level HCN), and 154 mg/day (high level HCN) HCN during the experiment.

Tissue Sampling

At the end of the experiment, all the animals were slaughtered and the samples of liver, thyroid, kidney, and thigh muscles (semimembranosus) were collected and stored at –20 °C for subsequent thiocyanate determination. Tissues section of liver were sliced to 3 mm and rapidly fixed in 10% buffered formalin for histological examination.

Chemical Analysis

Thiocyanate concentration in tissue samples were determined based on the colourimetric principle using the method proposed by Himwish and Saunders (1948). When ferric ions in acid solutions were added to thiocyanate, a brownish-red complex of ferric cyanate, with an absorption peak at 455 nm, was formed. This complex is a useful indicator of the amount of thiocyanate actually presents in the sample. Meanwhile, tissue samples to be assayed were homogenised and centrifuged at 5000 rpm. The clear supernatants were mixed with ferric nitrate reagent and further centrifuged before read at 455 nm using a spectrophotometer.

STATISTICAL ANALYSIS

Data of tissue thiocyanate were subjected to an analysis of variance (ANOVA), with the aid of SPSS 10 software (SPSS Inc., 1999). The full factorial analysis of variance was used to examine the effects of the treatment groups, as well as the species and their interactions. The difference between the treatment groups and species were tested according to the Duncan's Multiple Range procedure.

RESULTS

Thiocyanate Concentration

The concentrations of thiocyanate (SCN) in various organs of sheep and goats are shown in Table 1. Generally, the SCN concentrations in all the organs (except for the livers of both the treatment groups) were not significantly ($p>0.05$) different as compared to the control group. The

SCN concentration in the liver of goats HCN $_{7mg}$ group was found to be significantly ($p<0.05$) higher than the HCN $_{4mg}$ and the control groups. The same treatment (HCN $_{7mg}$) was given to the sheep, and the SCN concentration was only observed to be significantly different ($p<0.05$) from that of the control, but not with the HCN $_{4mg}$ group. Nonetheless, the SCN concentrations in the liver were not significantly ($p>0.05$) different between the species. The liver concentrations in both sheep and goats of the HCN $_{7mg}$ groups increased by about 20% and 30%, respectively, as compared to the control group.

Histopathology

The HCN $_{4mg}$ groups showed no pathological changes in relation to the control. The histological appearances of the liver in sheep and goats of the HCN $_{7mg}$ groups are presented in *Fig. 1*. Only the histological appearances of the goats are shown because of the similar degree of severity. There was demarcation of normal and necrotic cells. In some areas, necroses are centrilobular and the cells were shrunken.

DISCUSSION

Thiocyanate (SCN) is a less toxic product from the detoxification of hydrogen cyanide in the body and it can be used to determine cyanide loading related to the intake of cassava (Haque and Bradbury, 1999). The SCN concentration was significantly high in the liver of both sheep and goats which were fed with pelleted cassava leaves containing 154 mg HCN. The

TABLE 1
The mean thiocyanate (SCN) concentrations (µg/mL/g tissue) in various organs of sheep and goats in the different HCN treatment groups

Organs	Levels of HCN treatment						S.E.M	Between subjects-effects		
	Control		4 mg/kg HCN		7 mg/kg HCN					
	Sheep	Goats	Sheep	Goats	Sheep	Goats		Tr.	Sp.	Tr.*Sp
Kidney	3.83	3.33	4.51	4.27	5.00	4.34	0.24	NS	NS	NS
Liver	1.82^{c}	1.77^{c}	2.00^{b,c}	1.97^{b,c}	2.19^{a,b}	2.29^{a}	0.05	**	NS	NS
Muscle	1.90	2.18	2.81	2.67	2.69	2.01	0.20	NS	NS	NS
Thyroid	2.49	2.31	2.42	2.24	2.74	2.24	0.09	NS	NS	NS

^{a,b,c}: Means in the same row with different superscript/s differ significantly ($p<.05$)

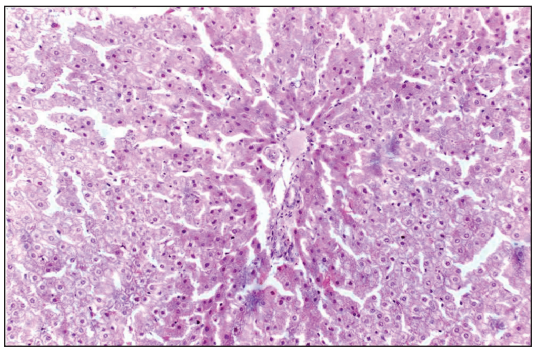

Fig. 1: Photomicrograph of the liver of goats in the HCN $_{7mg}$ group. Swelling and necrotic hepatocytes at the periportal area (arrow) [H&E, x100]

presence of high SCN content in the liver is due to the presence of high rhodanase enzyme (Westley *et al*., 1983) in the liver which is the primary site of detoxification of HCN to SCN (Oke, 1969). Although the levels of SCN in the kidneys of both the treatment groups were numerically higher, they were not significantly different as compared to the control group. Meanwhile, the low SCN content in other tissues could be explained by the fact that the SCN in the body occurred mainly in the tissue fluids such as blood, urine, saliva, gastric juice, and extracellular fluid (Smith, 1968). Thiocyanate penetrates the cellular membranes of different tissues at different rates. According to Moody (1971), the highest penetration has been found in anion concentrating tissues such as thyroid, salivary glands, gastric mucosa, and mammary glands. However, the non-significant finding of the SCN in thyroid gland in the present study was most probably due to the short experimental period.

Several pathological changes have been observed in the liver of sheep and goats, and these support the findings of the previous researchers'. Hepatic necrosis (Kamalu, 1993) was the similar lesion found in both animals of the HCN $_{7mg}$ groups. Periportal necrosis has also been referred to as peripheral lobular necrosis (Popp and Cattley, 1991). It may be evident grossly by its distinctive lobular pattern. However, it is extremely difficult to discern from the gross appearance whether the necrotic area is centrilobular or periportal. Several different reasons have been proposed for the periportal distribution of injury. The periportal area is the first area of the hepatic lobule to be exposed to a toxin being delivered via the blood stream (Huxtable, 1988). This suggests that the periportal hepatocytes may receive the largest dose of the toxin, since cells further down the sinusoid may be partly protected by removal of the toxicant in the periportal area. In some instances, the periportal distribution of hepatotoxicity is apparently due to metabolic zonation, including the greater oxygen tension in the portal than in the central lobular area of the lobule. It is important to note that a metabolic basis accounts for the distribution of centrilobular necrosis compared to periportal hepatocytes. Meanwhile, the centrilobular hepatocytes have much higher concentrations of cytochrome p-450 and associate enzymes (MacLachlan and Cullen, 1991). The distribution of the metabolising system, resulting in a higher concentration of the toxicant in the central lobular region, accounts for the occurrence of centrilobular necrosis. According to Solomonson (1978), liver cytochrome oxidase is not inhibited by cyanide, whereas brain may be the site of lethal action. This is in contrast with the finding of periportal necrosis in the liver. However, Kamalu (1993) demonstrated that the periportal vacoulation found in the liver of dogs fed garri was not due to the cyanide derived from cassava or metabolite thiocyanate. There was another factor detected in cassava, rather than cyanide, i.e. most probably linamarin (major cyanogenic glucosides in cassava) which was responsible for the lesion observed in the liver. However, the results gathered in the present study showed that the periportal necrosis found in the liver is related to the accumulation of thiocyanate in the liver of sheep and goats suggesting that the damage is probably due to presence of thiocyanate.

CONCLUSIONS

This study has shown ingestion of cyanide in cassava forages at high level HCN (7 mg HCN/ kg body weight) particularly in goats resulted in accumulation considerable amount of SCN, in which liver is the target organ for accumulation. In particular, cyanide in cassava fodder might exert a cytotoxic effect on metabolically active tissues such as liver. The effect caused cell death through the selectively poisonous actions of its metabolites cyanide (thiocyanate) which resulted in the necrosis of the liver. Therefore, it could be concluded that animals fed 7 mg HCN/kg body weight were unable to tolerate and affected pathologically.

ACKNOWLEDGEMENTS

This study was supported by the Intensification of Research Priority Areas (IRPA), Grant No. 01-03-03-0153 from the Ministry of Science, Technology and Innovation of Malaysia.

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