Onderstepoort J. vet. Res., 56, 145–146 (1989)

RESEARCH COMMUNICATION

THIAMINASE ACTIVITIES AND THIAMINE CONTENT OF *PTERIDIUM AQUILINUM, EQUISETUM RAMOSISSIMUM, MALVA PARVIFLORA, PENNISETUM CLANDESTINUM* AND *MEDICAGO SATIVA*

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ABSTRACT

MEYER, P., 1989. Thiaminase activities and thiamine content of *Pteridium aquilinum, Equisetum ramosissimum, Malva parviflora, Pennisetum clandestinum* and *Medicago sativa*. *Onderstepoort Journal of Veterinary Research*, 56, 145–146 (1989).

Thiaminase type 1 and 2 activities and thiamine content of five plants were determined. Of these *Pteridium aquilinum* and *Equisetum ramosissimum* were found to have considerably more thiaminase activity and lower thiamine content than *Malva parviflora, Pennisetum clandestinum* and *Medicago sativa*.

INTRODUCTION

Consumption of large amounts of plant material containing a thiamine (vitamin B1) destroying enzyme (thiaminase), may lead to cerebrocortical necrosis (CCN) in ruminants (Appling, Steele & Nottle, 1983). Under normal conditions, synthesis of thiamine in the rumen together with exogenous intake, is sufficient to meet the host's requirements (Brent, 1976). A plant often associated with CCN in Australia and the USA is bracken fern *(Pteridium aquilinum)* (Appling *et al.*, 1983). In South Africa, this plant has been associated only with bracken staggers, a nervous disorder of horses resulting from a thiamine deficiency, and a radiomimetric syndrome of cattle characterized by haemorrhagic tendencies and bone-marrow suppression, induced by a norsesquiterpene glucoside (Kellerman, Coetzer & Naude, 1988). Apart from bracken fern and horsetail *(Equisetum ramosissimum)*, kiesieblaar *(Malva parviflora)* may contain thiaminases (Kellerman *et al.*, 1988). It is entirely likely that other South African plants contain thiaminases, which may contribute to the occurrence of CCN in sheep grazing on natural pastures where known thiaminase-containing plants are absent (P. Meyer, Onderstepoort VRI, personal observation, 1988).

Two forms of thiaminase, types 1 & 2 (EC. 2.5.1.2 & EC. 3.5.99.2) have been identified (McCleary & Chick, 1977). Both are present in the rumen, where they are produced by ruminal bacteria (Boyd & Walton, 1977). Bracken fern, however, is reported to have only type 1 thiaminase activity (Everist, 1981). Thiaminase type 2 activity has not yet been found in plant material (McCleary *et al.*, 1977). Type 1 is a methyl transferase, which needs a co-substrate for the reaction to proceed, and type 2, a hydrolase, which is independent of a co-substrate (McCleary *et al.*, 1977). Although many *in vitro* co-substrates have been identified e.g. pyridine, niacine, proline, aniline, imidazole and cystein, the true *in vivo* co-substrates, both in plant and rumen fluid, are still open to question (Kennedy & McCleary, 1981. A likely co-substrate in rumen fluid, however, appears to be pyrroline, while in plant material it appears to be picoline (Edwin, Markson & Jackman, 1982).

Since a high concentration of endogenous thiamine in plants containing thiaminase may help to counterbalance the destruction of thiamine in the rumen, the thiamine content as well as thiaminase activity was determined in bracken fern, horsetail and kiesieblaar, and in kikuyu *(Pennisetum clandistinum)* and lucerne *(Medicago sativa)* as reference material.

Received 9 February 1989—Editor

MATERIALS AND METHODS

Approximately 1 kg of each plant was collected in the Pretoria district in November, air dried for ten days and the whole plant finely milled. Analyses were carried out in 4 separate trials. In each trial, the plant material was analysed in fourfold for thiamine content and thiaminase type 1 and 2 activities. The thiamine determinations were carried out according to the method of Edwin, Jackman & Hebert (1975), with the aid of a Shimadzu RF 540 spectrofluorimeter.

For the determination of the thiaminase type 1 and 2 activities, the principle of the method described by Rowe, Bodilla, Preston & Leng (1980), was used. Of the milled plant material, 1 g was extracted with 10 mℓ of a citrate-phosphate buffer (pH 6,4, 0,1 M) for 1 h at 39 °C under constant shaking. The plant-buffer mixture was used for the determination of the thiaminase activity. Thiaminase type 1 activity was distinguished from that of type 2 by the addition of 0,05 mℓ pyridine to the mixture as co-substrate. After incubation with radiolabelled (^{14}C) substrate, the solutions were centrifuged (2 500 *g*) for 30 min, and 2,5 mℓ of the supernatant was used to determine the release of labelled product and hence activity.

Thiaminase type 1 activity in bracken fern and kiesieblaar was also determined using aqueous solutions of aniline, proline, imidazole, niacine and cystein as co-substrates.

RESULTS AND DISCUSSION

The thiamine content and thiaminase activities of the plants are given in Fig. 1. The preferred *in vitro* co-substrates for thiaminase type 1 activity in bracken fern, in diminishing order, were aniline, pyridine, imidazole, proline, niacine and cystein. These differences however were slight, indicating that more co-substrate was added than necessary for type 1 enzymes to destroy the available thiamine. Virtually no thiaminase type 1 activity could be

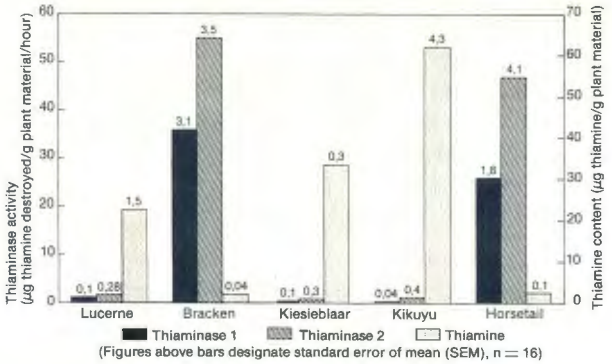

FIG. 1 Thiaminase activities and thiamine content of the investigated plants.

demonstrated in kiesieblaar with any of these *in vitro* co-substrates.

In the plants analysed in the current limited trial, high thiaminase activity was always associated with a low thiamine content. This may be due to a reduced rate of thiamine synthesis in these plants or it may result from a high rate of endogenous thiamine breakdown. In Australia, CCN is only seen in sheep grazing on natural pastures if great quantities of bracken fern are consumed (Everist, 1981). For this reason, it is postulated that this lesion is most likely induced by a high thiaminase activity coupled to a low thiamine content of the forage. This hypothesis may also hold true for the thiaminase containing plants in South Africa that lead to the development of CCN in sheep grazing on natural pastures.

The fact that all the plants displayed a higher type 2 thiaminase activity than type 1, suggests that thiaminase type 2 activity could well be present in the thiaminase containing plants but has previously escaped identification. A more likely possibility is that, if co-substrate was present in the plant, some type 1 activity may have contributed to that of type 2, thereby giving an inflated impression of the activity of the latter. This co-substrate was probably present in a limited amount, as the addition of further co-substrate gave rise to an increase in total thiaminase activity. The presence of such a co-substrate could be a major factor in controlling the level of thiaminase type 1 activity (McCleary *et al.*, 1977). If, as suggested here, *in vivo* co-substrate is present, it would be impossible, by using the thiaminase determination method of Rowe *et al.*, (1980), to distinguish between the two types of thiaminase activities.

The findings of this investigation should be interpreted with caution, as the stability of the thiaminase activities *in vitro* may differ considerably from that of the same thiaminase in the rumen. The possibility of thiaminase type 1 activity from a plant such as bracken fern being potentiated in the rumen by the simultaneous ingestion of a plant containing co-substrate should be investigated.

ACKNOWLEDGEMENTS

I am indebted to Dr T. S. Kellerman, Veterinary Research Institute, Onderstepoort, for valuable assistance in writing the manuscript.

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Printed by the Government Printer, Private Bag X85, Pretoria, 0001