THIAMINASE ACTIVITY IN THE RUMEN OF CATTLE GIVEN DIETS BASED ON SUGAR CANE, MOLASSES AND SISAL PULP[1]

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A method for estimating thiaminase activity in rumen fluid of cattle is given. The method is based on one published by Edwin and Jackson (1979). Thiaminase activity was found in the rumen fluid of all cattle examined and was at a high level in animals on a basal diet of molasses or derinded cane. Between 200 mg and 4.3 g thiamine per day could potentially have been degraded by bacteria in the rumen, suggesting a possible major involvement of thiamine in productivity responses to supplementation, of cattle on sugar cane, with vegetable proteins.

In cattle given a high molasses diet, thiaminase activity in the rumen was low and remained low following withdrawal of roughage from the diet up to the occurrence of symptoms of molasses toxicity six days later. The associated low rumen turnover rate, associated with this condition, together with low thiaminase activity, might affect the availability of thiamine from the digestive tract. Thiaminase activity was not associated with protozoa in the rumen nor was it present in the rind or pith of sugar cane.

Key words: Sugar cane, molasses, cattle, molasses toxicity, thiaminase, roughage intake.

Molasses toxicity is a condition that occurs sporadically in cattle fed a basal diet of molasses under feed lot conditions and it can be induced by withdrawal of forage from the diet. Rowe et al (1979a) and Lora et al (1978) demonstrated that, despite evidence for the disease being associated with decreased availability of glucose or glucose precursors (Losada and Preston 1973; Gaytan et al 1977), glucose entry rates remained high and constant during the course of induction of the disease by removal of forage from the diet. A finding in the studies of Rowe et al (1979a) was that the flow of fluid from the rumen decreased progressively following withdrawal of forage from the diet. When clinical symptoms of the disease appeared the flow of digesta from the rumen had almost ceased.

Molasses toxicity is accompanied by necrosis of cells in the central nervous system and is similar to a condition induced by lack of thiamine absorption. This occurs because the thiamine is degraded by a very active thiaminase produced in the rumen and intestines by bacteria (see Edwin et al 1979). If thiaminase is active in the rumen and if thiamine is relatively unavailable for absorption from either the rumen or the lower digestive tract because of the reduced movement of digesta and therefore thiamine to the lower gut, this could bring about the clinical symptoms of the condition. in order to study further this important disease condition the incidence of thiaminase in rumen contents of cattle on molasses diets from which the roughage was withdrawn

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has been examined. Thiaminase was found to be present in rumen fluid in all animals studied. Thiaminase activity in cattle on other diets based on sugar has also been studied.

Materials and Methods

Animals and treatments: The animals used to measure the effect of withdrawing the roughage from a molasses-based diet, on the level of thiaminase activity in the rumen, were four Zebu bulls weighing approximately 190 kg and were fitted with permanent cannuale in the rumen. The animals were given a diet consisting of molasses (+2,5% urea) ad libitum plus 8 kg cassava forage prior to the experimental period. For the final six days of the experiment only molasses/urea was given.

In the second part of these studies in which the thiaminase activity in the rumen fluid of cattle on a number of different diets was measured, a total of seventeen bulls (Zebu x Brown Swiss and Zebu x Holstein) with permanent rumen cannulae were used. Eleven of the animals were at the CEDIPCA in the Dominican Republic and six were at the University of Yucatan, Mexico. All animals were housed under cover in individual stalls The mean liveweight of these animals was 2}0 + 7 kg. All these animals were being used for other experiments and were given their rations in a single feed each morning. The diets are given in Table 3~together with the estimated level of thiaminase activity.

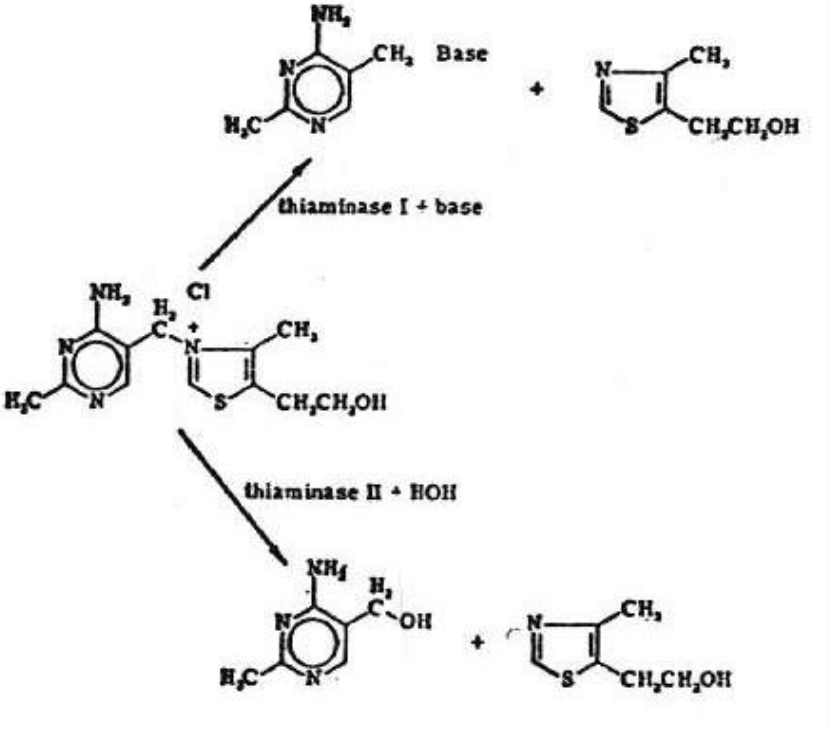
Procedures and measurements: In the four bulls given molasses/urea, without any roughage for the six day experimental period, samples were taken each day for the estimation of VFA. During this period, four measurements of rumen volume and turnover rate were made by injecting polyethylene glycol (PEG) intraruminally. The procedures and chemical methods used here were detailed in a previous paper (Rowe et al 1979a).

Estimation of thiaminase activity in rumen fluid:

Principle: A modification of the method of Edwin (1979) was used. The principle for its use is as follows: Two types of thiaminases are known. Both catalyze the breakdown of thiamine at the methylene bridge into thiazole and pyrimidine moieties(see Figure 1), Thiaminase I [thiamine: base 2-methyl-4-aminopyrimidine-5-methenyl transferase EC 2.5.1.2] is a transferase and requires a cosubstrate for the reaction to proceed. Aromatic primary amines, heterocyclic bases, or sulfhydryl compounds are known to function as cosubstrates or activators. In this study pyridine was used as the cosubstrate. Thiaminase II [thiamine hydrolase, EC 3.5.99.2] brings about an hydrolysis of thiamine at the methylene bridge. By using thiamine labelled in the thiazole moiety with ^{35}S, and measuring the amount of this compound formed in the reaction, the activity of the enzyme can be assayed. Further, the method can be used to differentiate between the two types of thiaminases.

The thiazole moiety is highly soluble in ethyl acetate, whereas thiamine is only slightly soluble. Therefore a rapid extraction of the thiazole into ethyl acetate and assay of its radioactivity following incubation of ^{35}S-thiamine with rumen contents, is a rapid method of assaying thiaminase activity.

Figure 1:
Summary of the action of the two known types of thiaminase (Edwin (1979)



Thiamine labelled with radioactive sulphur 35 in the thiazole moiety (i.e.^{35}S thiamine) was obtained from The Radiochemical Centre, Amersham, England.

Method: Into a test tube place 0.05ml of an aqueous solution of S thiamine (19 µg 150 mµc) together with 0.05 ml of an aqueous solution of pyridine (3.2 mg) which acts as the co-substrate. Rumen fluid (5 ml), which was collected from the animal immediately prior to the test, was added and the tube was placed in a water bath for 30 min at 39^{o}C. Ethyl acetate (10 ml) was then added and the tube was shaken vigorously 30 times by hand and then allowed to stand on the bench for 1 min for the aqueous and ethyl acetate layers to separate. The upper, ethyl acetate, layer was poured into a centrifuge tube and centrifuged at 3000 rpm for 5 min to remove any residual aqueous droplets. Two ml of the supernatant which was free of water droplets was placed in a scintillation vial together with 10 ml of Aquasol scintillation cocktail. The radioactivity was assayed in a Packard Scintillation Spectrometer Model B3255 using the charnels ratio method to adjust for quenching.

In all assays boiled rumen fluid treated in the same way as the fresh fluid was used as the control. In animals on four different diets samples were taken at zero time and after 30 and 60 minutes of incubation. Samples of the sugar cane pith, rind and of rumen protozoa were incubated in the same way to investigate if thiaminase activity was associated with these feed ingredients or micro-organisms.

Results

The results for the activity of thiaminase in rumen fluid were calculated as µg thiamine degraded to thiazole per hour per litre of rumen fluid (pg/hr/l). Typical results for the radioactivity extracted into ethyl acetate following incubation of rumen fluid from cattle on a number of diets with ^{35}S-thiamine are shown in Table 1. Approximately 1300 dpm (in 2 ml ethyl acetate) were extracted from boiled rumen fluid; with live rumen fluid the dpm extracted increased with time of incubation. It took approximately 2 min to prepare the samples and therefore the controls prepared at the commencement of incubation were assumed to have been incubated for 2 min.

The rate of increase in dpm between 30 and 60 minute incubation appeared to be relatively constant for samples taken from animals on different diets (35.8 ± 1.6% increase in final 30 minutes). Therefore for speed of analysis an incubation time of 30 minutes was decided on. No thiaminase activity was found to be associated with sugar cane or the rumen protozoa.

Table 1:
Typical thiaminase assay results obtained with rumen fluid taken from bulls given different diets.

	Incubation time (min)	Sugar cane (dpm)*	Cut grass dpm)	Derinded sugar cane (dpm)	Banana forage & molasses (dpm)
Boiled rumen fluid	-	1341	1274	1345	1444
Rumen fluid	2	2691	8788	1749	1730
" "	30	26458	26620	4114	3076
" "	60	36600	35420	5812	4081

* The results are dpm of 5 thiazole in 2 ml of ethyl acetate.

Effect of withdrawing forage from the diet on thiaminase activity and rumen function: The intake of molasses by the cattle following with drawal of roughage from the diet was reduced slightly but in general remained approximately 4 kg/d. As observed in a previous experiment (Rowe et al 1979a), the rate of rumen fluid turnover decreased markedly with time following withdrawal of roughage and there were no significant changes in the molar proportions of VFA in rumen fluid (Table 2). The thiaminase activity estimated during this experiment was low in all animals and did not show any tendency to change with time (Table 2).

Table 2:
The effects of removal fibre from the diet on the turnover rate of fluid, molar proportions of VFA and thiaminase activity.

Days after forage removal	Rumen fluid turnover rate (vol/d)	Percent VFAs as:				Thiaminase* activity µg/hr/l
		Acet	Prop	But	Others	
0	2.05	67	20	10	3	222 ± 68
1	1.82	70	20	8	2	358 ± 142
4	1/03	61	19	16	4	207 ± 10
5	0.69	67	20	10	3	241 ± 88

*Potential for breakdown of thiamine in µg degraded per hour per litre of rumen fluid.

[1] Disintegrations per minute

Thiaminase activity in the rumen: Thiaminase activity in the rumen fluid of cattle given grass or whole cane was higher than in those on derinded cane or molasses. Results for a number of the cattle have been calculated in terms of the potential breakdown of thiamine in the rumen (Table 3) and again the basal diets apparently affected considerably the estimated thiamine activity. On the grass and whole cane diets the thiaminase activity of rumen liquor was ten-fold higher than in cattle on derinded cane or molasses based diets.

In general the results obtained in the cattle in the Dominican Republic and in Mexico were similar. Low thiaminase activity was observed in the rumen fluid of all animals on the diets being used in Mexico except for one animal on a molasses based diet. In this animal there was a large population of 'window pane' organisms (Coleman G J pers, comm.), similar to the Methanosarcina bakerii found in the rumen fluid of sheep on molasses diets (Rowe et al 1979b).

Table 3:
Thiaminase activity in rumen fluid from cattle given a variety of diets based on tropical crops and byproducts.

Basal diet	No.of animals	Potential thiaminase* animals activity µg/l/hr		Potential** thiamine degradation
		Mean	Range g/d	
A Whole suger cane	4	2624	390-4936	2.5
B Derinded sugar cane	2	174	120-239	0.2
C Cut pasture	1	3700	-	3.6
D Molasses	4	222	0-490	0.2
E Molasses	6	981	201-3032	0.9
F Sisal pulp	2	559	95-1197	0.5

* Results are expressed as thiamine degraded per litre of rumen fluid per hour
** The results are extrapolated to y thiamine potentially destroyed per day in the rumen assuming a rumen volume of 40 1. Results for cattle on A to D were obtained in Santo Domingo; the results for cattle on dicta E and F were obtained in Mexico.
Diet A: 10 kg fresh chopped auger cane + 500 g cottonseed meal.
B: Derinded auger cane ad lib 10 kg sweet potato forage, 750 g cottonseed meal.
C: 10 kg fresh cut pangola grass + 10 kg sweet potato forage.
D: Molasses ad lib (+ 2½% urea) + 15 kg chopped banana stem.
E: Molasses ad lib. (+ 2½% urea) + 4 kg cane stalk.
F: Sisal pulp ad lib + 7 kg leucaena.

Discussion

The major objective of the research was to examine the possibility of bacteria that produce thiaminase, multiplying to excessively high numbers in the rumen under conditions of low rumen turnover as occurs when animals suffer molasses toxicity. However thiaminase activity was low in cattle on molasses/urea diets prior to removal of roughage from the diet and remained low through to the onset of clinical symptoms. The extent of the potential thiamine breakdown represented 200-400 mg/d if the in

vitro results are extrapolated to the in vivo situation. This is well below the extent of breakdown that occurs in ruminants with classical cerebro-cortical necrosis on grain based diets (Edwin and Jackman 1974) where the potential for thiamine destruction is of the order of several grams per hour (Edwin et al 1979). However, it must be recognised that on the diets used in this study, little or no dietary thiamine is ingested and therefore the animal is dependent upon synthesis by micro-organisms in the rumen and/or lower digestive tract. This synthesis might be much less than the estimated potential degradation rate of thiamine by ruminally produced thiaminase. Thiamine content of the rumen rarely exceeds a total of 30 mg in cattle and Hungate (1966) suggested that only 8.8 mg/d of thiamine left the rumen of a sheep given a grass diet; this shows that the thiamine availability could be much less than the potential for thiamine degradation. This together with the low turnover rate of rumen fluid and thus the low flow rate of digesta (and therefore thiamine) to the lower digestive tract might be sufficient to cause the animal to become deficient in thiamine. The thiamine requirement of a 360 kg animal has been estimated to be approximately 16 mg daily (Losada et al 1971),

Contrary to these results, Mella et al (1976) could not demonstrate any thiaminase activity in rumen fluid of cattle on molasses based diets. In addition,tissue levels of thiamine in cattle with molasses toxicity were similar to that in control animals. This, however, does not rule out the suggestion that all animals on this diet may be marginally deficient even when tissue levels are apparently normal (Lehninger 1978). The rapidity of onset of the disease (ie 5 days after removal of the roughage) suggests that the animal might be precariously balanced for thiamine even when roughage is present in the diet. The results presented here suggest that it is possible that a low thiaminase activity in the rumen coupled with low flow rates of material out of the rumen induce a thiamine deficiency in animals which could eventually result in a cerebral necrosis as indicated by the clinical symptoms of molasses toxicity. At this stage we have not been able to examine animals over an extended period of time to obtain estimates of the fluctuations in thiaminase activity but obviously the possibility of marginal thiamine deficiency on some of these diets cannot be disregarded.

There are persistent references to high blood glucose levels in animals with molasses toxicity; for instance Geerken and Figueroa (1971) found that the average blood glucose levels in affected animals were 87.5 mg/100 ml as against 51.4 mg/100 ml in control animals. The high blood glucose levels in the studies of Lora et al (1978) were not however associated with an increased entry rate of glucose and thus there appears to be a decrease in glucose utilisation. This again would be accounted for by a marginal deficiency in thiamine. Another aspect of animals on molasses which supports the concept of marginal thiamine deficiency is their apparent reduced ability to lay down fat; again if thiamine was marginally deficient then the pentose phosphate pathway of glucose metabolism would be decreased (this is because thiamine pyrophosphate is an essential co-factor in the functioning of the pathway) and this may result in a lack of reduced NADP which is necessary for long chain fatty acid synthesis.

Of major significance was the finding of high thiaminase activity in the rumen of the four cattle given diets of whole sugar cane and cottonseed meal. Of these animals, one gave consistently low thiaminase activity but in the other three animals the potential thiamine breakdown in the rumen could have been of the order of 3 to 4 g/d. With this potential for thiamine breakdown in the rumen it appears that the animal could have insufficient thiamine available to meet its requirements. However

Bobadilla et al (1978) were not able to demonstrate any responses to B vitamin supplementation on whole cane diets supplemented with meat and bone meal and given B vitamin sources in the diet or by injection. These studies repeated earlier work of Lora and MacLeod (1976) who were also unable to demonstrate a growth response to supplementation with B vitamins on sugar cane diets supplemented with cottonseed meal. However dose rates of B vitamins were low relative to recent therapeutic doses.

These results have important implications for feeding trials and might explain to some extent the variability in the response to supplements of proteins and forages given to cattle on basal diets of sugar cane or molasses. The effectiveness of the supplements may partly be due to their increasing the supply of thiamine to the animal, either directly in the form of bypass dietary B-vitamins (eg cottonseed meal, rice polishings) or indirectly by increasing the turnover rate of rumen fluid, thereby improving the supply of thiamine of microbial origins, to the animal (eg sweet potato and cassava forages). It is possible that a deficiency of thiamine could override the useful effect on animal performance of feeding proteins which are not completely fermented in the rumen.

Further work is necessary to investigate the pattern of change of thiaminase activity in the rumen with time and also to examine the influence of specific microorganisms.

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