MOLASSES TOXICITY IN CATTLE: RUMEN FERMENTATION AND BLOOD GLUCOSE ENTRY RATES ASSOCIATED WITH THIS CONDITION[1]

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Molasses toxicity is a condition characterized by irreversible brain damage, and in this it is indistinguishable from cerebrocortical necrosis (CCN) or encephalamacia. Molasses toxicity has occurred when cattle with free access to molasses (3% urea) have been given severely restricted amounts of roughage. In this experiment, 6 Zebu bulls were housed 3/pen. The animals weighed between 237 and 292 kg, and were fitted with permanent rumen cannulae. The animals were given a daily ration consisting of 11 kg sweet potato forage (17% DM) and 4.2 kg molasses (32 urea; 70% DM) for 6 weeks prior to and for the first 2 d of the experiment. For the last 9 of the experiment only molasses/urea was given, ad libitum. The daily feed intake of each group of animals was estimated each day. On 8 of the experimental days the following parameters were estimated: rumen fluid turnover rate and volume (using PEG); rumen fluid VFA concentration, and the molar proportions of each VFA; and blood glucose entry rate. On the 11th day, two animals were slaughtered in order to take samples of brain, liver and kidney for clinical and histopathological examination. When the roughage was removed from the ration, the turnover rate (volumes/d) of the rumen fluid decreased from 1.7 to 0.05, which in turn was associated with an increase in rumen volume from 32 to 75 litres. No significant changes or trends were observed in the daily consumption of molasses or in the estimation of rumen fluid VFA (approximately 90 mmol/litre: 69% acetic, 15% propionic, 15% butyric and 1% valeric acid). There was neither a significant change in the plasma glucose concentration, 69 ± 7 mg glucose/ 100 ml plasma (X, SEx), nor in the glucose entry rate, 819 ± 57 g glucose/d. Several areas of the cortex of the brain of each animal slaughtered had well defined areas of necrosis. All the an la showed the signs of the early stages of molasses toxicity: salivating, shivering and standing with the head down in a 'dejected' pose.

It was concluded that brain damage may occur during molasses toxicity without glucose being a, limiting nutrient. It was suggested that the very slew turnover rate of rumen fluid may be an important factor in this condition through its effect on the supply of protein and thiamine to the animal. The possible importance of the animal's body reserves (glucose precursors and thiamine) in buffering the animal against anoxic brain damage was discussed.

Key Words: Molasses toxicity, cattle, glucose, rumen turnover

In feeding systems where cattle are given molasses ad libitum and restricted amounts of roughage, a condition known as molasses toxicity may occur. The clinical syndrome and the encephalopathy arising from molasses toxicity have been found to be indistinguishable from those of cerebrocortical necrosis (CCN), also known as polioencephalomalacia (see Verdura and Zamora 1970; Edwin et al 1978). However, in this paper the term "molasses toxicity" will be used specifically to distinguish those studies which have been made on animals given molasses-based diets.

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[1] This work was financed by funds from the UNDP/FAO project DOM/77/002

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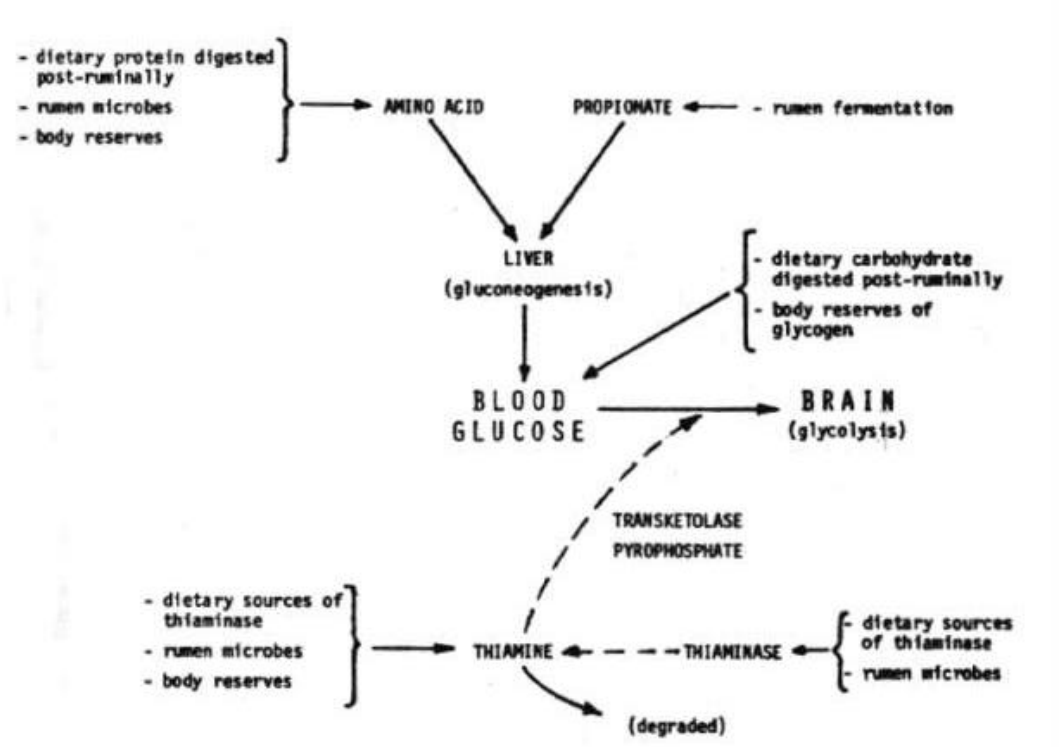
Figure 1 :
A summary which may affect the supply of blood glucose and its utilization by the brain



It seems likely that the immediate cause of necrosis in all of these conditions is through a decrease in the energy supply to the brain (Edwin et al 1978), and in Figure 1 an attempt has been made to summarize the factors associated with the supply of glucose and its utilization by the brain. A deficiency of thiamine, either through dietary inadequacy or through the action of thiaminase, has been shown in all cases to be the cause of CCN (Pill 1967; Edwin and Jackman 1973; Evans et al 1975; Edwin et al 1978). However, in studies on molasses toxicity, large amounts of thiamine given intra-ruminally or intra-muscularly were not effective in preventing the disease (Losada et al 1971) and this has led to the suggestion that the problem was due to a deficiency of glucose (Losada and Preston 1973a). In a recent study, Gaytan et al (1977) demonstrated that glycerol, given orally, at the rate of 400 g/d, was effective in preventing the disease condition, and it was suggested that this was because additional glucose was available from the post-ruminal digestion of the carbohydrate source. It is also well known (see Preston and Willis 1974) that if the signs of the disease are recognised early enough, complete recovery may be affected by restricting the animal's access to molasses and providing pasture ad libitum. Conversely, the condition may be induced by removing the roughage component from the ration of animals accustomed to consuming high levels of molasses and a roughage.

In the present study, the entry rate of blood glucose and the concentration of volatile fatty acids (VFA) in the rumen were estimated before, and for 9 days after the

roughage component was removed from a ration based on molasses In this way, the availability and utilization of glucose were measured as the disease condition advanced. The availability of propionate as a precursor of gluconeogenisis and the effect of a roughage-free diet on rumen fluid turnover rate, were also measured to assist in understanding the aetiology of the condition.

Materials and Methods

Animals and Treatments: Six Zebu bulls weighing between 237 and 292 kg were fitted with permanent rumen cannulae two months before the experiment and were housed 3/pen. The animals were given a daily ration consisting of 11 kg sweet potato forage and molasses containing 2.5% urea (w/w) ad libitum for 6 weeks prior to and for the first 2 d of the experiment. For the final 9 d of the experimental period, only molasses/urea was given.

All animals were prepared with catheters in the jugular vein on Day 1 of the experiment for injecting glucose tracer and taking blood samples. It was anticipated that these catheters would last the duration of the experiment. However, because the only catheters available were very fine, there were several instances when catheters became blocked. When this occurred, the catheter was removed and a new one inserted in the opposite side in order to carry out an injection and sampling period on the following day. For this reason, there were not estimations of blood glucose entry rate for each animal on each measurement day. The number of animals sampled each day is shown in Table 1. In animals 1 and 3, an infection began to develop around

Table 1:
Schedule of the days on which each estimate was made showing the number of animals sampled on each occasion

Estimation	Days of experiment										
	1	2	3	4	5	6	7	8	9	10	11
Rumen fluid volume and turnover rate	6	6	6	6	5	-	-	2	4	4	-
Rumen VFA	6	6	6	6	6	-	-	4	4	4	-
Blood glucose concentration	-	6	6	2	4	6	-	4	4	-	4
Glucose entry rate	-	6	4	1	4	1	-	4	4	-	4

Differences in the numbers of animal arise through occasions on which insufficient samples were taken and analysed to provide an accurate estimate of the rate of tecline in tracer concentration, or when catheters became blocked.

the jugular veins on Day 7, and these animals were immediately withdrawn from the experiment, treated for the infection and given cut pasture ad libitum. The rumen fluid volume and turnover rate were measured in these two animals until the end of the experiment, but these estimations have not been included in any tables or figures in this paper.

Procedure and Measurements: The experimental period was 11 d. During this period, estimations were made of: the concentration and the molar proportions of the major rumen VFA; blood glucose entry rate; and, rumen fluid volume and turnover rate. The days on which these estimate. were made, and the number of animals sampled on each day are summarized in Table 1.

Polyethylene glycol (Carbowax 4000) (PEG) was used as a marker of rumen fluid and was injected intraruminally (100 g PEG in approximately 300 ml water) at 11 am. Five samples of rumen fluid (10 ml) were taken during the subsequent 24 hr at times approximately corresponding to 2, 4, 6, 22 and 24 hr after injection. A sub-sample (2 ml) was taken from each of these 5 samples and bulked for the analysis of VFA. These bulked samples were acidified and stored at -5°. Blood glucose entry was estimated by injecting intravenously approximately 200μ Ci [2- 3H] glucose (in 2 ml of boiled saline, 0.09% w/v) and then taking 6 samples of blood (10 ml) during the subsequent 3 hr at 30 min intervals. The samples of whole blood were immediately centrifuged (2000 rpm for 10 min) and the plasma taken and stored at -5° for analysis.

Feed intakes were recorded and samples taken for analysis of DM. The animals were closely observed for physical signs of molasses toxicity.

At the end of the experiment (Day 11), two animals (5 and 6) were slaughtered and samples of brain, kidney and liver taken for clinical and histopathological examination. These samples, preserved in both alcohol and formalin, were sent to the Central Veterinary Laboratory, Ministry of Agriculture Fisheries and Food, Weybridge, England, where they were examined for similarities with tissues of animals with CCN (see Edwin et al 1978).

Chemical Methods: The concentration of PEG in rumen fluid was estimated by the turbidametric method of Malawar and Powell (1967). Total VFA concentration was estimated by titration after steam distillation of 1 ml of rumen fluid using a Markham still. Approximately 150 ml solution was collected during distillation for titration. Following titration, excess alkaline was added before the sample was concentrated by boiling, and then dried at 65°C. Separation of the major rumen VFA (acetic, propionic, butyric and valeric acids) was by gas-liquid chromatograph. The dried sample was acidified using 0.05 ml orthophosphoric acid solution (5 molar) and 1-4ul injected into the column. The details of the column that was used are as follows: 1.25 m x 2 mm (internal diameter) stainless steel; column temperature: 125°C; inlet temperature: 200°C; column packing: Supelco, mesh #60/80 coated with Tween 80. Hydrogen was the carrier gas (22 ml/min) and a flame ionization detector (Carle Instrument Company; Model 311) was used in conjunction with a recorder (Omniscribe Company; Houston) to measure the molar proportions of VFA relative to a standard solution which was injected after every 6 samples. Compressed air (20 ml/min) was mixed with the carrier gas before the burner to increase the sensitivity of detection.

Glucose concentration in plasma was estimated by the glucose oxidase method of Hugget and Nixon (1957). Glucose was isolated from plasma as the penta-acetate derivative (Jones 1965) for assay of radioactivity with 10 ml of toluene, containing 0.4% (w/v) PPO and 0.02% (w/v) POPOP, as the scintillant.

Calculations: The plasma glucose pool and the rumen fluid were both considered to be single compartments and it was assumed that there was no recycling of either tracer, [2--H] -glucose or PEG, during each sampling period. The mathematical

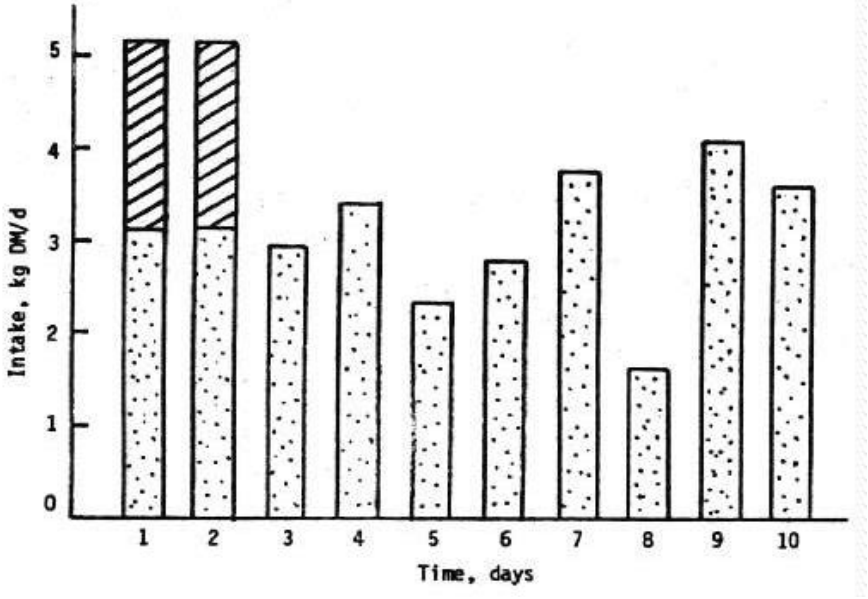
considerations of analysing this type of system have been described previously (eg Shipley and Clark 1972). To correct for the background of PEG in rumen fluid continuing from the infusion of the previous day, the 24 hr intercept for the regression relationship of Ln (PEG concentration) against time was substracted from the zero time intercept of the same relationship determined for the following day, to give the value from which the rumen fluid volume was estimated. Any regression relationship of Ln (PEG concentration) against time which had a value of $r^2 < .90$ was not analysed further.

A blood sample to estimate the specific radioactivity (SR) of plasma glucose was not taken before each injection of radioisotope. Therefore, only the zero time intercept of the decay in glucose SR with time (measured after the first injection of [2-3H] -glucose) was used to estimate the plasma glucose pool size. From this pool size and the concentration of glucose in plasma, measured in each animal on Day 2, a 'glucose space' was estimated for each animal. This value was used to estimate the plasma glucose pool size on subsequent days by multiplying the glucose space by the mean glucose concentration estimated for each animal. The turnover rate (d) was estimated from the regression coefficient in the relationship between Ln (glucose SR) against time measured for each injection of radioactivity.

Results

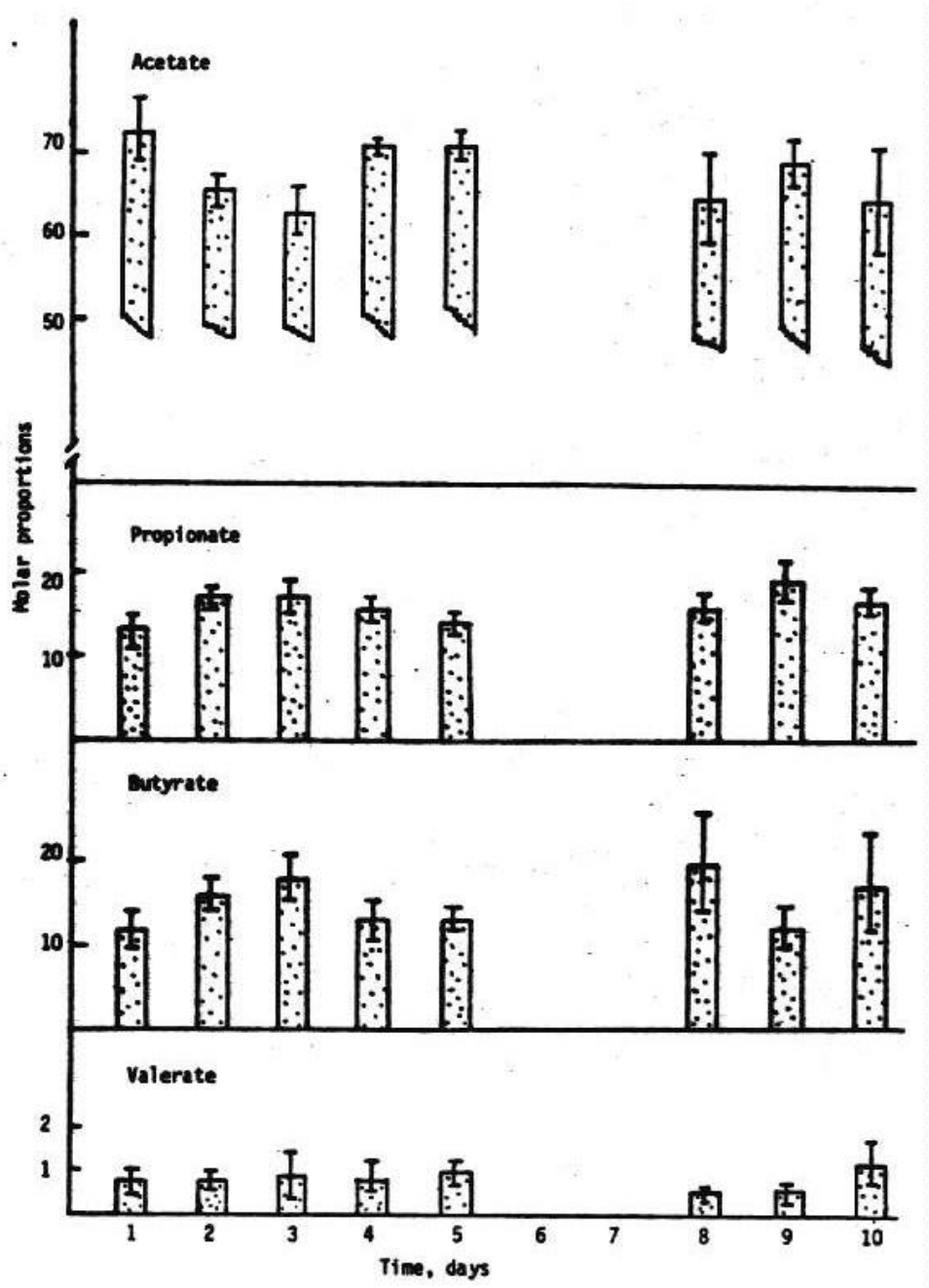
The daily consumption of molasses (69% DM) and of sweet potato forage (17% DM) is shown in Figure 2. There was no significant change in molasses intake when sweet potato forage was removed from the ration during the experiment. The concentration of VFA in the rumen fluid did not vary between animals or during the

Figure 2:
Intake of molasses (□) and of sweet potato forage (■). Means of 6 animals until Day 6, then means of 4 animals.



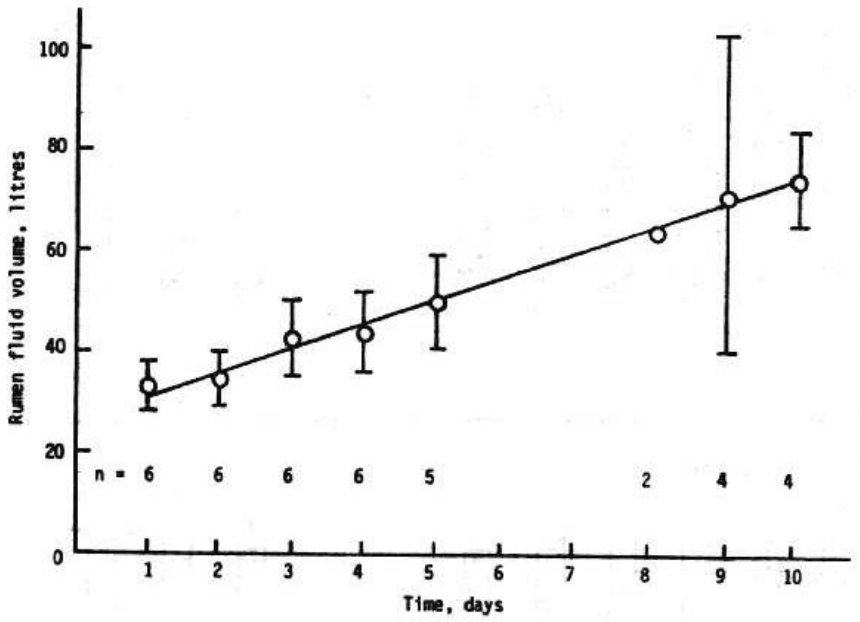
experiment: 90 ± 3 mmol/litre (x, SEx). The molar proportions of the major VFA are shown in Figure 3; there were no trends with time for any of the VFA.

Figure 3:
The molar proportions of the four major VFA (X,SEx); valterate and iso-valerate are shown as a single histogram



The mean rumen volume increased from 34 litres, when sweet potato forage was in the ration, to 75 litres, after 8 d during which only molasses was given (Figure 4). There was a large amount of variation in rumen volume both within and between animals. The rate of rumen fluid turnover decreased from approximately 1.7 volumes/d

Figure 4:
The change in rumen fluid volume with time after removing the roughage component of the ration. Points show: X, SEx

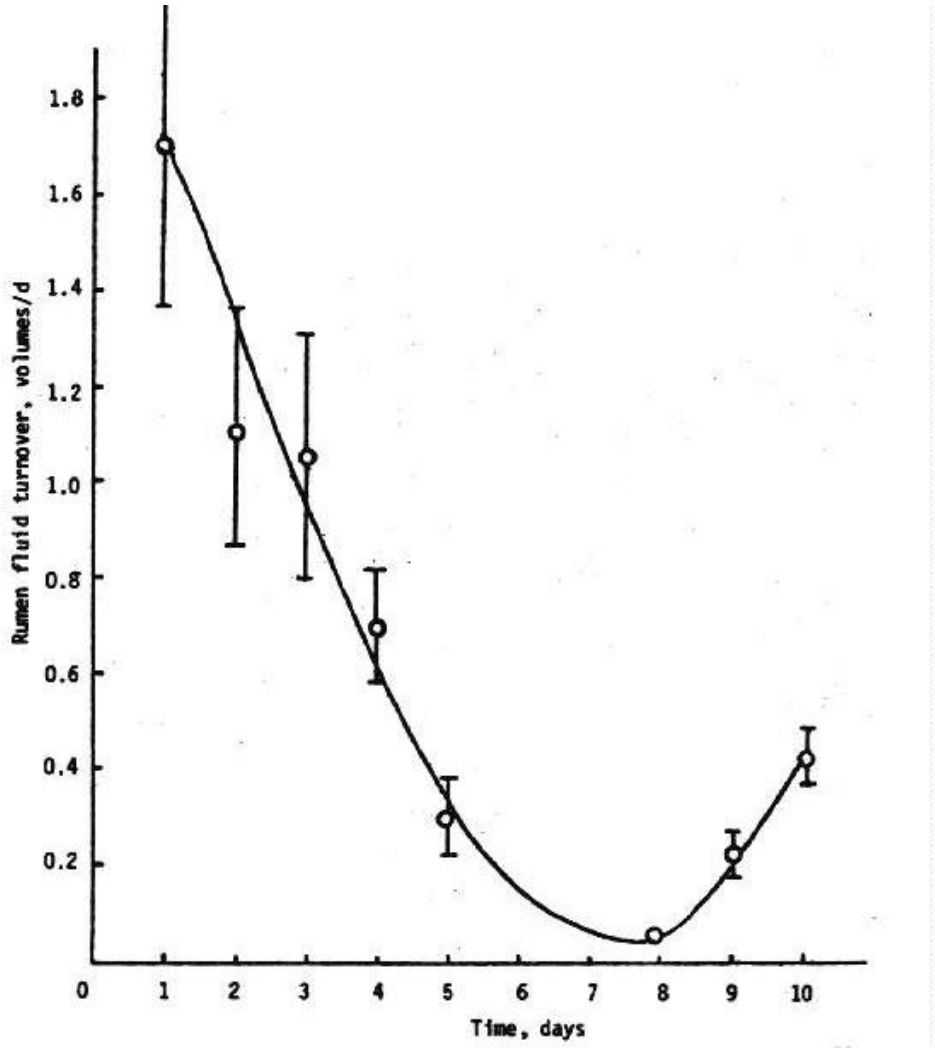


when forage was present in the ration to 0.05 volumes/t after 6 d when only molasses was given (Figure 4). The change in the rate of fluid flow from the rumen is shown in Figure 5. A decrease in flow rate with time is clear. The rumen fluid turnover rate estimated in animals #1 and #3 which were given cut pasture ad libitum and no molasses after Day 7 increased from an average 0.38 volumes/d on Day 5, to 1.28 volumes/d on Day 10. There was little variation in the plasma glucose concentration within or between animals during the experiment and no significant changes in glucose entry rates with time (Figure 6). In animal #5, there was a reduction in glucose entry rate from 739 g glucose/d on Day 8 to 91 g glucose/d on Day 11 (Figure 7). Animal weights measured on Day 5 of the experiment were 292, 364, 280, 291, 237 and 245 kg for animals #1 to #6 respectively.

From Day 7 of the experiment, after 5 d without roughage, all animals showed the signs characteristic of the early stages of "molasses toxicity". These were: shivering, salivating, and standing with the head down in a 'dejected' pose. These signs were not observed in all animals at all times but were seen in each animal for varying periods each day, It was not possible to grade the animals in order of the seriousness of the disease from the frequency and intensity of the periods of shivering and salivating.

Post-mortem examination of the brain of animals #5 and #6 showed several

Figure 5:
The change in the rate of rumen fluid turnover with time after removing the roughage from the ration. Points show: x, SEX



areas of the cortex with distinct yellow colouration where necrosis had occurred. The Central Veterinary Laboratory, Weybridge, also found evidence of necrosis of the brain of the same type observed with CCN, but pathological and biochemical diagnoses were not conclusive (the tissue was damaged during collection and transportation).

Discussion

It is clear that during this experiment the animals suffered brain damage and also that this necrosis did not reach an advanced stage. The animals showed definite behavioural signs of molasses toxicity and areas of necrosis were clearly visible in the post-mortem examination of the brains of two of them. There are, therefore, two main

Figure 6:
Plasma glucose concentration (X, SEx) estimated over the period of experiment

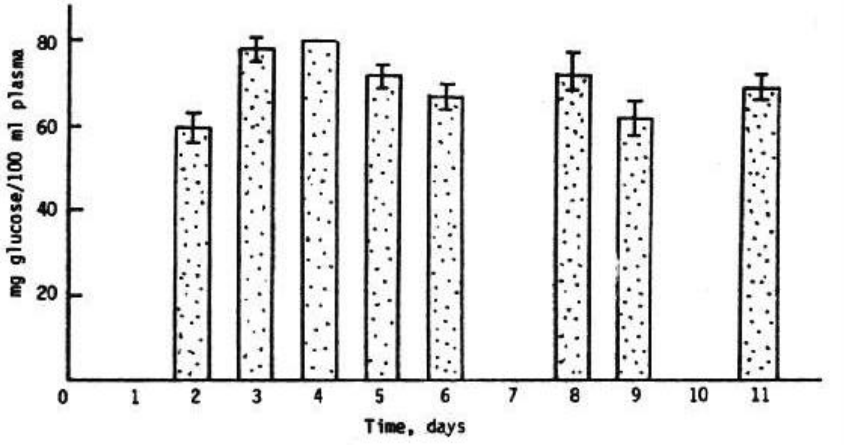
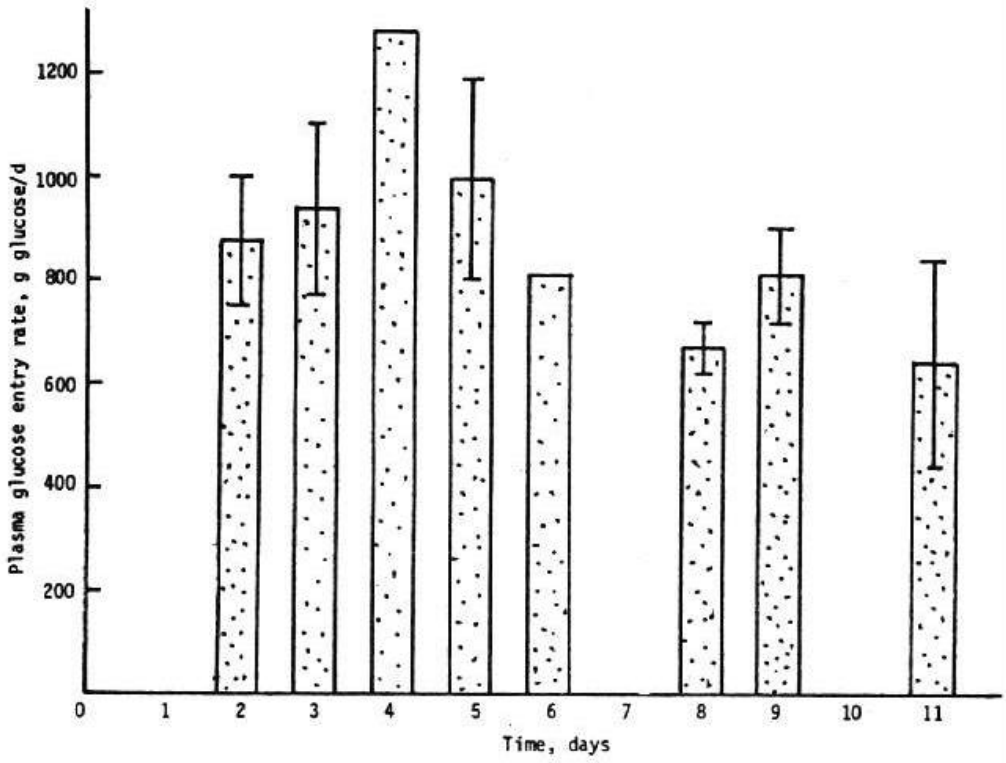


Figure 7:
Plasma glucose entry rates (X, SEx) estimated over the period of the experiment



areas for discussion: (I) why the signs of molasses toxicity in this experiment were less severe than anticipated from the results of other studies, and (ii) why the necrosis occurred.

(i) From the results of previous experiments (Losada et al 1971; Losada and Preston 1973b; Gaytan et al 1977) and from past experience with this condition, it was expected (a) that the animals would have suffered from severe intoxication between 1-10 d after the roughage was removed from the ration, and (b) that there would have been a marked fall in rumen propionate concentration, The animals used in this experiment were in very good condition, having completed a 3 month feeding trial in which molasses and sweet potato forage were given and during which time the mean daily rate of liveweight gain was 750 g/d. In experiments reported by Losada et al (1971), Losada and Preston (1973b) and Gaytan et al (1977), the animals used were considerably lighter. In addition, to this it is clear from discussions with local farmers that the incidence of molasses toxicity has been found more commonly in situations where molasses is given to cattle in poor condition, following a period of low pasture availability caused by drought, It is therefore suggested that the condition of the cattle may be an important factor determining their susceptibility to molasses toxicity, Animals in good condition might have sufficient reserves of glucose precursors and/or thiamine to act as a 'buffer' to prevent anoxic damage to the brain. This is summarized in Figure 1.

Losada and Preston (1973b) found that the concentration of rumen propionate was greatly reduced when forage was absent from the ration, and hypothesised that the low availability of this nutrient as a precursor for gluconeogenesis caused the interruption in the supply of energy to the brain. We were not able to detect an effect on rumen VFA in this experiment. That the concentration of propionate remained relatively high would also serve to reduce the severity of the disease condition, if glucose availability is a factor.

(ii) In this experiment, neither glucose entry rate nor blood glucose concentration were affected by removing the forage component from the ration of animals consuming high levels of molasses. This is consistent with the data of Lora et al (1978) in which relatively high glucose entry rates were observed in severely 'intoxicated' animals. It is therefore suggested that the brain damage observed may have resulted from the blocking of the pentose phosphate pathway due to a deficiency of the thiamine-containing enzyme, TPP, as described by Edwin (1978) for CCN. In two previous studies (Losada et al 1971; Mella et al 1976), it did not appear that thiamine was involved in the aetiology of the molasses toxicity. However, there is no reason for attributing the occurrence of CCN to any single factor. In fact, as discussed by Edwin (1978) and summarized in Figure 1, it is likely that a variety of factors acting either individually or synergistically may contribute to the occurrence and the severity of the necrosis. Many studies have been carried out in ruminants on the effects of starvation (eg Blaxter 1966), and although animals have had no feed for periods of 1-3 weeks, no cases of CCN have been reported. This indicates that a fall in the availability of glucose precursors from dietary sources does not per se result in brain damage.

The most obvious effect associated with the removal of forage in this experiment was the rapid reduction in the turnover rate of the rumen fluid (from 1.5 to 0.05

volumes/d) and it seems that this may be one of the central factors associated with the aetiology of the disease. The increase in rumen volume associated with the very low flow of liquid from the rumen was also observed by Losada and Preston (1973b). One result of rumen stasis would be an abrupt fall in the supply of both protein and B-vitamins to the animal, associated with the interrupted flow of microbial cells from the rumen. In a ration based on high levels of molasses/urea, very little true protein is provided and the supply of protein and B-vitamins to the animal from microbial synthesis is of greater importance than when forage or cereal grain provide the main constituents of the ration. In molasses/urea feeding systems, there is characteristically a good response in liveweight gain to the inclusion in the ration of a high quality animal or oilseed protein (see Preston 1972). It may be hypothesized, therefore, that if protein supplied principally by microbial synthesis in the rumen is insufficient for optimal animal performance, then the B-vitamins supplied from the same source may potentially be a limiting factor, and that an interruption in their supply through rumen stasis may produce deficiency symptoms such as CCN.

The observation that forage given to animals #1 and #3 from Day 7 rapidly increased the rate of rumen fluid turnover, together with the fact that supplying forage to animals has been the only effective method of treating the disease (Preston and Willis 1974), lends support to the suggestion that the rumen fluid turnover rate may be of principal importance in this condition. It still has to be explained how glucose metabolism by the brain can be inhibited, while overall glucose entry rates were unchanged,

Conclusions

In this study, early signs of "molasses toxicity" and brain damage were observed without any evidence of glucose being a limiting nutrient. It was suggested that in this experiment the energy supply to the brain was interrupted by a deficiency of the enzyme transketolase pyrophosphate blocking the metabolism of glucose. This is in contrast to the hypothesis that the disease is caused by a reduction in the supply of precursors for gluconeogenesis which has developed from indications that (I) thiamine may not be deficient in cases of molasses toxicity (Losada et al 1971; Mella et al 1978); (ii) glycerol given in the ration (as a source of glucose available to the animal) serves to protect against outbreaks of the disease (Gaytan 1977); and (iii) the concentration of rumen propionate may be low when no forage is given in the ration (Losada and Preston 1973b).

It therefore appears that the aetiology of the disease may involve a number of factors acting either individually or synergistically. Clearly, it is necessary to measure simultaneously the availability and utilization of glucose, the availability of thiamine, and the thiaminase activity in the rumen during the development of the condition, to understand the way in which these factors interact to reduce the supply of energy to the brain.

The authors are grateful to Dr E E Edwin and his colleagues at the Central Veterinary Laboratory, Weybridge, England for their examination and analysis of the tissue samples. The assistance of M de J Peralta in cathetizing the animals, and that of A Hernandez and J J Sepulveda in training the animals for sampling is gratefully acknowledged. This work was initiated after an interesting and stimulating discussion with Professor R H Dunlop.

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Received 4, April 1979