REACTION OF RATS FED ON LEUCAENA LEUCOCEPHALA

B A El-Harith, Y Schart and U ter Meulen

Institut fur Tierphysiologie und Tierernahrunf -der Universitat Gottingen
Oskar-Kellner-Weg 6, Weende, 3400 Gottingen, West Germany

Growing rata consiming diets containing 25% Leucaena leucocephalla meal (equivalent to 0.722 mimosine in the diet) suffered from mild alopecia. cataracts, reversible paralysis of the hind limbs, severe retardation of growth and mortality. Whereas addition of ferrous sulphate (2%) to the ration protected the animals from developing these toxic symptoms; dry heat treatment of the Leucaena meal (at 90~C for 20 hrs) failed to produce a similar result.

Key Words: Rats, feeding, leucaena, mimosine

Leucaena leucocephala is a leguminous shrub belonging to the family mimosaceae, Its original habitat was Central America, but it is now widely grown in most tropical regions of the world, This plant has high potential value as forage for live-stock, because of its high protein content and palatability. The use of the plant as a forage is however limited by the toxic amino acid mimosine which may induce various adverse reactions in animals (Meulen et al 1979).

Many reports have discussed the deleterious effects caused in rats by feeding them on Leucaena meal or on mimosine (Yoshida 1944; Yang and Ling 1968; Tsai and Ling 1974; Hegarty et al 1976; Grove et al 1978). Despite the abundance of literature on mimosine toxicity, many aspects of these toxic reactions are not fully understood It is also necessary to search for treatments which would render the Leucaena meal a fodder which causes no ill effects. The present study was aimed at investigating the effects of feeding Leucaena leucocephala untreated, heat-treated or supplemented with ferrous sulphate to young rats.

Materials and Methods

Dried seeds and leaves of Leucaena leucocephala were obtained from Zaire, and after milling (0.5 mm sieve) were mixed in the ratio 1:1 by weight to form a Leucaena meal.

The mimosine content of the urine and faeces samples was determined by the method of Hegarty et al (1964). Mimosine (used as a standard) was isolated from the seeds of the plant and recrystallised by the method of Spenser and Notation (1962).

Weanling wistar Albino male rats of an SPF-derived colony and mean body weight of 50 grams were used in this study. The animals were caged singly and assigned to 4 groups of 9 animals each. They received diets as described in Table 1.

Table 1 :
Diets of rats used in experiment on the effect of pretreatment of Leucaena on mimosine toxicity

Group	Control	LM	LM + $FeSO_4$	HLM
Standard rat's ration (control diet)	95%	75%	73%	75%
Leucaena meal (LM)	0	25%	25%	0
Heated Leucaena meal (HLM)[1]	0	0	0	0
Ferrous sulphate	0	0	2%	0
Fish meal	5%	0	0	0

[1] Heated Leucaena meal = dry Leucaena meal that was heated in an oven at 90°C for hrs.

The standard ration was supplemented with fish-meal in order to raise the raw-protein content to 19%, which was the protein level in all the test diets of this experiment (Analysis of raw-protein by Kjeldahl).

The animals were kept for 5 weeks. At the end of the second week, 4 animals from the group fed Leucaena meal (LM) and heat-treated Leucaena-meal (HTLM)were transferred to the standard control diet for the remaining 3 weeks of the trial.

All feeds were provided ad libitum. Recording of body weight was performed weekly. Urine and faeces were collected daily and stored at 2! C prior to the analysis of their mimosine content.

Results

A very mild loss of hair (alopecia) was noticed in the animals fed Leucaena meal (LM) and animals fed Heat treated Leucaena meal (HLM). The animals were not bald and the hair could not be readily pulled from the skin.

Paralysis of the hind-limbs of all the animals of the LM and HLM groups was clearly noticeable on the 10th day of the experiment. However, when some of these animals were then fed on the standard diet, their hind limbs regained their normal function after about 5 days,

Cataracts were noticed in the animals of HLM groups after 3 weeks. Cataracts were also noticed on the 21st day in the animals which were switched from LM and HLM diets to the standard diet.

Animals of LM and HLM groups suffered from dramatic retardation of growth and noticeable distress which was sometimes accompanied by diarrhoea.

No deaths were recorded amongst the animals on the control diet or amongst those switched back to the standard diet after two weeks. However, in group LM one animal died in each of the 2nd, 3rd and 4th experimental weeks. Also in group HLM 2 animals died in the 2nd week and one in the 3rd week.

Upon gross autopsy of the dead animals no apparent injury of the internal organs was readily detectable.

A remarkable impairment of growth was clearly noticeable in those rats of LM and HLM treatments. Animals of the FeSO4 treatment showed moderate retardation of growth relative to controls. Animals switched back to the standard diet quickly

increased in body weight so that at the end of 5 weeks their body weight approached that of the control group (Table 2). The feed intakes of the animals of LM and HLM

Table 2:
Mean body weight of growing rats fed on a standard ration or on different rations containing Leucaena leucocephala meal

Group	Control	LM	Reversed from LM	LM + $FeSO_4$	HLM	Reversed from HLM
Start:	50	50	[1]-	49	50	-
End of expt. Week:						
1	68	50	-	60	46	-
2	84	47	47	69	43	43
3	104	51	78	81	38	85
4	118	58	99	94	43	101
5	147	68	128	113	49	123

1 - = not determined
2 LM = Leucaena meal diet
3 HLM = heated Leucaena meal diet

treatment were considerably reduced relative to controls, especially in the third and fourth week. The feed intakes of the other experimental groups were not seriously affected (Table 3).

It was observed that the mean faecal weight of animals of the LM + $FeSO_4$ treatment was considerably higher than that of the control animals. The mean faecal weight of animals of the HLM treatment, on the other hand, was noticeably lower than that of the control animals (Table 3).

Table 3:
Feed intake (DM)[1] and weight of faeces (DM) of rats receiving different diets containing Leucaena meal.
(all values in g/rat/week)

	Feed Intake						Faecal weight					
Expt. Week	Control	LM	Reversed from LM	LM + $FeSO_4$	HLM	Reversed from HLM	Control	LM	Reversed from LM	LM + $FeSO_4$	HLM	Reversed from HLM
2	55	29	-	46	25	-	6.5	2.7	-	13.5	-	3.6
3	52	7	38	47	8	55	8.7	5.0	8.5	11.2	6.9	3.2
4	53	14	56	51	12	57	7.6	8.8	8.3	13.2	8.5	3.3
5	58	35	41	56	20	54	7.1	7.6	7.8	11.3	6.9	3.8

[1] DM - Dry matter

Animals on LM and HLM treatments had higher concentrations of mimosine excreted in their urine than those of the LM + FeSO treatment. Those animals reversed from Leucaena diets to standard diets showed a gradual decrease of mimosine in the urine as expected (Table 4),

Table 4:
Excreted mimosine in the urine and faeces of rats receiving different diets containing Leucaena meal

	Feed Intake						Faecal weight					
Week	Control	LM	Reversed from LM	LM + $FeSO_4$	HLM	Reversed from HLM	Control	LM	Reversed from LM	LM + $FeSO_4$	HLM	Reversed from HLM
2	0	90	-	9	-	-	0	17	-	.63	18	-
3	0	80	10	5	107	17	0	6	2	13	7	3
4	0	171	11	17	95	5	0	17	0	25	23	3
5	0	113	5	30	66	4	0	74	0	44	33	0

Discussion

The results presented here show some adverse affects induced in young rats consuming leaves and seeds of Leucaena leucocephala,

Although this experiment was not specifically designed to determine the LD50 of mimosine, it seems that the dose which the animals received i.e. 0.72% mimosine in the diet, was approximately equal to the LD50 , since 3 out of 5 animals died in groups receiving untreated and heat-treated Leucaena meal.

L-tyrosine was found to have completely counteracted the growth inhibition in rats caused by mimosine (Yoshida et al 1944) whereas phenylalanine could only moderately compensate this growth inhibition (Lin et al 1964). It is possible that the palatability of feeds containing Leucaena meal is rather poor in rats, This is evidenced by the very low feed intake of LM and HLM groups especially in the 3rd and 4th week of the experiment (Table 3). The relative increase in the feed intake in the 5th week could possibly be due to some adaptation of the animals to their diets. The low feed intake was to some extent reflected in the very poor weight gain up to the end of the 4th week (Table 2). Therefore, it is thought that the retardation of growth of our rats could be explained partly by the reduced feed intakes.

Cataracts were observed to be irreversible, and in this respect, reference is made to the work of Sallmann et al (1959), who reported a reduction in the mitotic index of lens epithelial cells in mimosine induced cataracts.

Paralysis of the hind limbs in rats consuming Leuceana meal diets was, unlike cataracts, reversible upon withdrawal of Leucaena meal from the diet of the animal. With the exception of one report (Yoshida 1944), no other worker on the toxicity of Leucaena leucocephala has reported a similar finding. It is probable that the alkaloid mimosine was responsible for causing paralysis of the hind limbs in rats. Further work on the neurotoxi city of mimosine is recommended.

Mimosine was either absorbed from the gastrointestinal tract and then excreted in the urine or excreted directly in the faeces. Accumulation of mimosine in the serum and eyes was evidenced by the fact that both groups or animals reversed from Leucaena diets continued to excrete mimosine up to the 4th week and also developed cataracts on the 20th day of the experiment. Similar evidence for the accumulation of mimosine in the skin, eyes and serum of the rat were reported by TSAI and Ling (1974).

We noticed that animals feeding on LM diet supplemented with ferrous sulphate excreted in the faeces amounts of mimosine which were of the same order of magnitude or slightly greater than other groups consuming Leucaena meal. However, the concentration of mimosine in the urine of the animals from this group was very much lower than that of the animals of LM and HLM groups. This observation can be explained by the fact that mimosine was chelated by iron FeSO4 in the LM + FeSO4 diet (Tsai and Ling 1973), and hence it was not easily absorbed from the gastrointestinal tract and was excreted mainly in the faeces.

Paper chromatography of the urine samples from all the animals feeding on Leucaena meal diets did not confirm the presence of any metabolises of mimosine in the urine. This was not unexpected as non-ruminants, unlike ruminants, are incapable of hydrolysing mimosine into 3,4-dihydroxypyridine (Hegarty et al 1964).

In this study it was apparent that addition of FeSO4 to the ration containing Leucaena meal was effective in protecting our animals from developing the toxic symptoms due to mimosine. Similar results were obtained with growing chicks (Labadan 1969). Heating the Leucaena meal at 90!C for 20hr prior to its incorporation in the diet was not effective in rendering it non-toxic to our animals. It has been found by other workers that moist heating over 70!C of Leucaena meal was effective in protecting the animals consuming Leucaena meal from the toxic effects due to mimosine (Matsumoto et al 1951). It is believed that mimosine is hydrolysed at elevated temperatures, and it was our intention to see if dry heating could lead to a similar result as moist heating, as dry heating could have been of a more practical value.

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