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Fate of Mimosine Administered Orally to Sheep and its Effectiveness as a Defleecing Agent

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Abstract

Mimosine was administered orally to Merino sheep once daily for periods of 1–3 days, either as the isolated compound or in the foliage of *Leucaena leucocephala*. A single daily dose of mimosine of 450 or 600 mg/kg body weight was effective for defleecing sheep. A daily dose rate of 300 mg/kg was effective for defleecing sheep if given on two sucessive days.

The effectiveness of a treatment for defleecing sheep was related to the concentration of mimosine in plasma following dosing; defleecing ensued when the concentration of mimosine in plasma was maintained above 0·1 mmol/l for at least 30 h.

The main products excreted in urine were mimosine and 3,4-dihydroxypyridine (DHP); small amounts of mimosinamine were also excreted. During the first day following dosing, the major excretory product was mimosine; DHP was an important component during the second and third days. In the three days following the start of dosing, between 32 and 53% of the mimosine given was accounted for as mimosine in the urine.

Following an intravenous infusion of mimosine, no DHP was detected in urine; most of the mimosine was excreted intact but a small amount (*c.* 9%) was excreted as mimosinamine.

Introduction

The amino acid mimosine (β-[*N*-(3-hydroxy-4-oxopyridyl)]-α- aminopropionic acid), which is present in appreciable amounts in the seeds and foliage of *Leucaena leucocephala*(Lam.) de Wit, is a depilatory agent but is also toxic to sheep (Hegarty *et al.* 1964*b*). Reis *et al.* (1975) showed that mimosine was a satisfactory defleecing agent for sheep, without any apparent side effects, if it was infused intravenously for 1½–2 days at the daily rate of *c.* 80 mg/kg. Under these conditions the concentration of mimosine in plasma rose rapidly to about 0·2 mmol/l, wool growth stopped within 2 days and the sheep were subsequently defleeced with ease.

Hegarty *et al.* (1964*b*) found that the intraruminal infusion of mimosine into a sheep, at an average daily rate of 170 mg/kg for 8 days, failed to cause shedding of the fleece. Indirect evidence was obtained that mimosine was extensively degraded to 3,4-dihydroxypyridine (DHP) in the rumen under these conditions (Hegarty *et al.* 1964*b*). However, shedding of the fleece did result from the sustained feeding of rations incorporating sun-dried *L. leucocephala* leaf which contained mimosine (Hegarty *et al.* 1964*b*), and it was concluded that a daily intake of mimosine of 200–300 mg/kg body weight was necessary to produce shedding.

Based on the results of Hegarty *et al.* (1964*b*) after feeding sheep on *L. leucocephala*, Reis *et al.* (1975) suggested that oral administration of mimosine for short periods

might be a satisfactory means of defleecing sheep. Accordingly, mimosine was administered orally to sheep once daily for periods of 1–3 days, either as the isolated compound or in the foliage of *L. leucocephala*. The effectiveness of mimosine as a defleecing agent, its concentration in blood plasma, and the excretion of mimosine and its metabolites in urine following dosing, were investigated. It was found possible to defleece sheep with a single oral dose of mimosine.

Materials and Methods

Sheep and Diet

Merino wethers, weighing approximately 30 kg, were kept indoors in metabolism cages or single pens, and were fed individually once daily at about 10 a.m. The daily ration was 600 g of a ground and pelleted mixture of lucerne (3 parts) and oats (2 parts). Drinking water was available *ad libitum*.

Administration of Mimosine

Sheep were dosed orally with mimosine in one of two ways:

(i) Mimosine was isolated from seeds of *L. leucocephala* (Beyerman *et al.* 1964; Hegarty and Court 1964), and was placed in gelatin capsules (3·0 or 4·5 g per capsule). The sheep were dosed once daily, with two or more capsules depending on dose, directly into the rumen using a balling gun. Capsules were found to disintegrate in rumen contents in 6–8 min, so the sheep were observed for at least 10 min after dosing to ensure that the capsules were not regurgitated before they had disintegrated.

(ii) New growth was collected from *L. leucocephala* plants, and was dried for 24 h at 50°C in a forced-draught oven. The dried leaf material was separated from the stems and was found to contain 4·4% mimosine on an air-dry basis. A ground and pelleted diet was prepared consisting of *L. leucocephala* leaf-meal (2 parts), oats (1 part), and lucerne (1 part). During the experimental feeding period the basal diet was replaced by an amount of the above diet (410 g on two successive days; 820 g on one day) to supply the required amount of mimosine (Table 1). The diet was offered once daily.

Mimosine was infused intravenously using procedures described by Reis *et al.* (1975).

Plan of Experiments

Experiment 1. Twelve sheep were dosed orally with mimosine daily in amounts ranging from 200 to 600 mg/kg body weight for periods of 1–3 days, as indicated in Table 1.

Experiment 2. Six sheep were given sufficient of a diet containing *L. leucocephala* leaf-meal to provide mimosine at the daily rate of 300 mg/kg for 2 days or 600 mg/kg for 1 day (see Table 1).

Experiment 3. One sheep was given an intravenous infusion of mimosine at the daily rate of 120 mg/kg (i.e. 4 g/day) for 2 days.

Analysis of Mimosine and Metabolites in Plant Material, Blood Plasma and Urine

Ground *L. leucocephala* leaf was extracted with 0·1M HCl as described by Hegarty *et al.* (1964*a*). Blood samples were collected from the jugular vein at intervals after dosing, using heparin as an anticoagulant. Plasma was separated by centrifugation and the samples were stored at −10°C pending analysis. The concentration of mimosine in the leaf extract and in blood plasma was measured as described by Reis *et al.* (1975).

The complete output of urine from some sheep was collected daily for 3 or 4 days, and was stored at −10°C pending analysis. No preservative was added. The concentration in urine of mimosine and its metabolites (mimosinamine [*N*-(2-aminoethyl)-3-hydroxy-4-pyridone], free DHP and conjugated DHP) was measured by a modification of the method of Hegarty *et al.* (1964*a*); full details of the procedure will be reported separately. The modified procedure involves elimination of the treatment of the urine with a cation-exchange resin, and the use of high-voltage, thin-layer electrophoresis on cellulose to separate the various metabolites, and microspectrophotometric estimation of the compounds as the ferric complexes. The method can be used to detect amounts in the range of 1–5 μg.

It was demonstrated that mimosinamine was not formed in urine after voiding, as none was detected during incubation of mimosine with freshly voided urine for 48 h at 25°C. Mimosinamine was identified in urine on the basis of the following evidence. It had the same mobility as authentic mimosinamine (Takahara and Takahashi 1972) on thin-layer ionophoresis on cellulose at pH 2, 3·5, 7, and 9, and gave the same colour reactions with ninhydrin and ferric chloride reagents. It emerged in the same position (after arginine) as the authentic material when chromatographed in a Beckman Multichrom B amino acid analyser under the conditions used to separate basic amino acids.

Results

Effectiveness of Oral Administration of Mimosine for Defleecing Sheep

The effectiveness of oral administration of mimosine for defleecing sheep depended on the rate and the duration of administration (Table 1); it was equally effective when given as the isolated compound or as *L. leucocephala* leaf-meal. A dose of 200 mg/kg, given on 3 successive days, did not cause defleecing. A single dose of 300 mg/kg was effective for defleecing only one out of two sheep but, when given for 2 or more days, this dose rate was effective for all sheep so treated (Table 1). Higher levels (450 and 600 mg/kg) were effective for defleecing sheep when given as a single dose.

Table 1. Effectiveness of oral doses of mimosine for defleecing sheep

Sheep were dosed once daily into the rumen with mimosine in gelatin capsules (expt 1), or were given a diet containing *L. leucocephala* leaf-meal (expt 2)

Expt No.	Mode of administration of mimosine	Mimosine dose (mg/kg body weight) Daily	Total	Duration of administration (days)	No. of sheep dosed	No. of sheep defleeced
1	As isolated mimosine	200	600	3	2	0
		300	300	1	2	1
		300	600	2	2	2
		300	900	3	2	2
		450	450	1	2	2
		600	600	1	2	2
2	As *L. leucocephala* leaf-meal	300	600	2	3	3
		600	600	1	3	3

Concentration of Mimosine in Plasma

The concentration of mimosine in plasma was measured 6, 24, 30, and 48 h after the first dose of mimosine was given (expt 1), or after feeding on the first day (expt 2). The effectiveness of a treatment for defleecing sheep was related to the concentration of mimosine in plasma following dosing. Single daily doses of amounts of mimosine that were effective for defleecing sheep (450 and 600 mg/kg) produced a high initial concentration of mimosine in plasma and maintained a concentration above 0·1 mmol/l for about 30 h (Fig. 1*c*). Likewise, daily dosing with 300 mg/kg for at least 2 successive days defleeced the sheep and maintained plasma levels of mimosine above about 0·1 mmol/l for 30 h (Fig. 1*b*). In contrast, doses of mimosine that were not sufficient to cause defleecing (200 mg/kg daily, and 300 mg/kg for 1 day only) did not maintain plasma levels of mimosine above 0·1 mmol/l for a sufficient period (Fig. 1*a*). Effective doses of mimosine given as *L. leucocephala* leaf-meal resulted in levels of mimosine in blood plasma similar to those found with equivalent oral doses of mimosine (Fig. 1*d*).

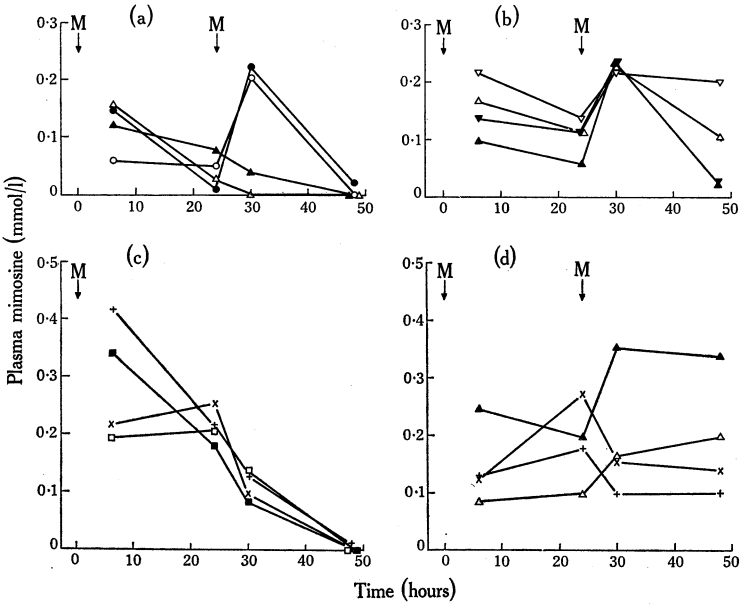

Fig. 1. Concentration of mimosine in blood plasma following oral doses of mimosine. Times of mimosine dosing are indicated by M. (*a*) Four sheep from experiment 1 received mimosine doses of (i) 200 mg/kg at 0 and 24 h (○, ●), and (ii) 300 mg/kg at 0 h only (△, ▲). (*b*) Four sheep from experiment 1 received mimosine doses of 300 mg/kg at 0 and 24 h. (*c*) Four sheep from experiment 1 received mimosine doses of (i) 450 mg/kg at 0 h (□, ■), and (ii) 600 mg/kg at 0 h (+, ×). (*d*) Four sheep from experiment 2 received *L. leucocephala* leaf-meal which provided doses of mimosine of (i) 300 mg/kg at 0 and 24 h (△, ▲), and (ii) 600 mg/kg at 0 h only (+, ×).

Table 2. Excretion of mimosine and its metabolites

Details of the experiments are given in Table 1. Each sheep received a total of 18 g mimosine. The values for DHP include both free and conjugated DHP, expressed as free DHP. For calculation of the percentage of the dose excreted, the amounts of DHP and mimosinamine excreted were converted to equimolar amounts of mimosine

Expt No.	Treatment	Sheep No.	Mimosine excreted (g) on day 1	2	3	DHP excreted (g) on day 1	2	3	Total mimosinamine	% dose excreted in 3 days: As mimosine	As DHP	As mimosinamine	Total
1	Mimosine in capsules												
	300 mg/kg on	7075	2·43	3·08	0·26	0·70	1·90	2·35	0·32	32	49	2	83
	days 1 and 2	7249	2·59	3·95	0·38	0·22	2·03	1·93	0·96	38	41	7	87
1	Mimosine in capsules												
	600 mg/kg on	7096	4·17	2·04	1·89	0·48	0·84	3·19	0·66	45	45	5	94
	day 1	7217	7·50	1·68	0·41	0·20	2·74	0·73	0·21	53	36	2	91
2	*L. leucocephala* leaf												
	300 mg/kg on	7241	2·32	3·28	2·19	0·14	2·09	0·99	0·99	43	32	7	82
	days 1 and 2	7226	2·70	2·09	2·19	0·57	0·95	2·19	0·85	39	37	6	82
2	*L. leucocephala* leaf												
	600 mg/kg on	7244	2·20	4·09	0·82	0·63	1·01	0·23	1·89	40	19	14	72
	day 1	7232	1·81	3·54	0·81	0·33	0·55	0·41	0·42	34	13	3	50

Excretion of Mimosine and its Metabolites

The pattern of excretion of mimosine and its metabolites following oral dosing was measured in eight sheep. Daily urinary outputs are given in Table 2 for mimosine and for free plus conjugated DHP (expressed as free DHP), as well as the total output of a minor component identified as mimosinamine. Regardless of the dose rate or the

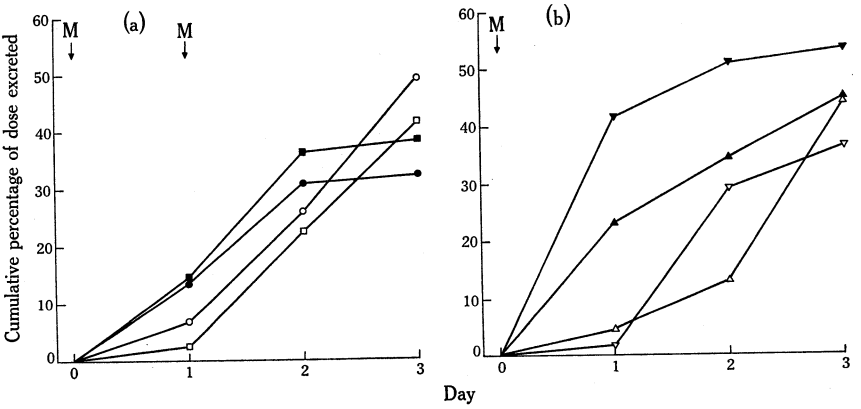

Fig. 2. Excretion of mimosine and DHP. The cumulative percentage of the dose excreted is shown for four sheep from experiment 1 (see Table 1) that received a total of 18 g mimosine (600 mg/kg). A different symbol is used for each sheep; excretion as mimosine (●, ■, ▲, ▼), excretion as DHP (○, □, △, ▽). (*a*) Two consecutive daily doses of mimosine (9 g) were given to two sheep as indicated by M. (*b*) A single dose of mimosine (18 g) was given to two sheep as indicated by M.

form in which it was given, mimosine was the major component excreted during the first day following dosing (Table 2). DHP was an important component excreted during the second and third days. Mimosinamine was a significant excretory product especially in one sheep that received *L. leucocephala* leaf-meal. The pattern of excretion following dosing with mimosine in capsules (expt 1) was influenced by the dosing

Table 3. Excretion of mimosine and mimosinamine following intravenous infusion of mimosine

A sheep received a continuous intravenous infusion of a total of 8 g mimosine over a period of 2 days. The total mimosinamine excreted (0·55 g) is equivalent to 0·71 g mimosine

	Day 1	Day 2	Day 3	Day 4	Total
Mimosine excreted (g)	2·34	3·01	0·83	0·16	6·34
Mimosinamine excreted (g)	0·24	0·20	0·07	0·04	0·55

procedure. A single dose of 600 mg/kg resulted in a greater excretion of mimosine on the first day, and in a slightly higher proportion of the dose being excreted as mimosine, than was obtained with a daily dose of 300 mg/kg given for 2 days (Table 2, Fig. 2). When mimosine was given in the form of *L. leucocephala* leaf-meal, the pattern of excretion was more variable and a smaller proportion of the dose was excreted within three days (Table 2).

Following an intravenous infusion of mimosine (4 g/day for 2 days), no DHP was detected in the urine and most of the mimosine infused was excreted unchanged in the urine during the first 3 days (Table 3). In addition small amounts of mimosinamine were excreted (Table 3). During the 4 days from the start of infusion, 88% of the mimosine given was accounted for in the urine; 10% of this amount was excreted as mimosinamine.

Discussion

These experiments have demonstrated that it is possible to defleece sheep after giving a single oral dose of mimosine. As the number of sheep dosed was small, no firm estimate can be made of the amount of orally administered mimosine required to cause defleecing consistently. However, a tentative estimate can be made that the minimum amount needed as a single dose is between 300 and 450 mg/kg body weight. This should be contrasted with a minimum amount of *c.* 120 mg/kg (80 mg/kg per day for $1\frac{1}{2}$ days) required when a continuous intravenous infusion of mimosine is given (Reis *et al.* 1975). Thus, at least three times as much mimosine would be needed for an oral dose compared to an intravenous infusion. It is apparent that mimosine given orally has a low activity as a defleecing compound compared with cyclophosphamide, which is effective at a dose rate of 25–30 mg/kg (Hourihan *et al.* 1970; Hohenboken 1972; Reynolds *et al.* 1972). The relative activities of mimosine and cyclophosphamide are also widely different when considered on a molar basis, as the molecular weights are approximately in the ratio of 1 : 1·3 (mimosine : cyclophosphamide). A relatively large amount of orally administered mimosine (of the order of 20 g) is required to defleece a sheep of average size. Thus, a more active derivative of mimosine would be desirable, or a form that is protected from degradation by the microorganisms in the rumen.

Reis *et al.* (1975) showed that, to defleece a sheep, sufficient mimosine needed to be infused intravenously to maintain a concentration of mimosine in blood plasma of 0·1–0·2 mmol/l for about 36 h. While the level of mimosine in blood was measured only infrequently in the current experiments, the data indicate that defleecing occurred only when the concentration of mimosine in plasma was maintained above about 0·1 mmol/l for at least 30 h after dosing. It would therefore appear that sufficient mimosine was absorbed from the gastrointestinal tract, following oral dosing, to account for the defleecing effects observed; it is not necessary to postulate that DHP or mimosinamine are active as defleecing agents. Neither of these compounds has been tested for activity in defleecing sheep. Indirect evidence (Hegarty *et al.* 1964*b*) suggests that DHP is probably not active as a depilatory agent for sheep. Mimosinamine is a depilatory agent for mice but has a lower activity than mimosine (Takahara and Takahashi 1972); it may be active in sheep. However, as judged by the amounts excreted in urine, its quantitative significance in our experiments is small.

During the first day after the commencement of oral administration of mimosine the main excretory product was mimosine; DHP made a significant contribution from the second day. This result is also in accord with the conclusion that sufficient mimosine escapes degradation in the rumen after oral administration to cause the depilatory effects. Since only 32–53% of the dose given could be accounted for as mimosine excreted in 3 days, it could be inferred that 2–3 times as much mimosine would be needed with oral dosing as with intravenous infusion to obtain

defleecing. However, as a high proportion of mimosine was excreted on the first day, the differential between the two routes of administration could be slightly greater.

Following the intravenous or abomasal administration of mimosine no DHP is excreted in the urine (Hegarty *et al.* 1964*b*, and the present study). When mimosine is given orally it must therefore be degraded to DHP by the microorganisms in the rumen. However, it is apparent that the first daily dose of mimosine given orally, either as the isolated compound or in *L. leucocephala* leaf-meal, is not extensively degraded to DHP. This may be related to a lag in the synthesis of adaptive enzymes required for degradation of mimosine, or to an inhibition of microbial activity by the high concentration of mimosine initially present in the rumen. Whatever the reason, this effect should always occur in practice if sheep were dosed only once a year to achieve defleecing.

The presence of mimosinamine in the urine of sheep following administration of mimosine or the feeding of *L. leucocephala* has not previously been observed. The excretion of mimosinamine following intravenous infusion of mimosine indicates that it is a product of metabolism of mimosine by the body tissues. Control experiments have shown that it is not formed as a result of bacterial action in the urine after voiding. This result does not preclude the possibility that some mimosinamine is formed by the action of rumen microorganisms following oral dosing.

In future work it will be neccessary to study larger numbers of sheep to determine more accurately the dose rate needed to obtain consistent defleecing, and to ascertain the extent of individual variation in response. Also, the possibility of adverse effects must be considered (Reis *et al.* 1975).

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