https://doi.org/10.1079/BJN19960111 Published online by Cambridge University Press

Effects of mimosine administered to a perfused area of skin in Angora goats

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(*Received 11 November 1994 – Revised 15 March 1995 – Accepted 11 May 1995*)

The effect of mimosine on a perfused area of skin tissue was studied using an isolated perfusion technique. Four mature Angora wethers (body weight 35 (SE 2·3) kg) were cannulated bilaterally with indwelling silicone catheters in the superficial branches of the deep circumflex iliac artery and vein. Mimosine (40 mg/kg metabolic weight ($W^{0.75}$) per d) was infused intra-arterially into one iliac artery of each goat for 3 d and saline was infused in the contralateral (control) iliac artery. Iliac venous blood samples were taken from both sides along with arterial samples from the carotid artery. Mimosine infusion elevated plasma mimosine in the carotid artery (52·6 (SEM 19·21) μmol/l) and iliac vein on the saline-treated side to 54·1 (SEM 16·31) μmol/l and in the iliac vein on the mimosine-treated side to 191·3 (SEM 19·14) μmol/l ($P < 0.01$). Mimosine decreased feed intake (2·3 *v.* 0·6 kg/d, SEM 0·29; $P < 0.001$) and water consumption (5·2 *v.* 1·3 litres/d, SEM 0·67; $P < 0.001$). Mimosine did not cause defleecing in the area of infusion and was cleared from the bloodstream within 12 h of cessation of infusion. The following effects were also observed during mimosine infusion: decrease in plasma amino acids to half pre-infusion values (methionine 22·7 *v.* 13·1 μmol/l, SEM 1·41; lysine 95·9 *v.* 37·4 μmol/l, SEM 4·28; $P < 0.001$); decreases in plasma triiodothyronine (1495 *v.* 695 ng/l, SEM 43·1; $P < 0.001$), thyroxine (61·5 *v.* 19·5 μg/l, SEM 1·8; $P < 0.001$) and insulin (28·7 *v.* 17·3 μIU/ml, SEM 1·89; $P < 0.01$) concentrations; increase in plasma cortisol (14 *v.* 62 μg/l, SEM 0·35; $P < 0.001$) concentration; decreases in levels of plasma Zn and Mg (0·97 *v.* 0·49 mg/l, SEM 0·063; $P < 0.001$ and 21·4 *v.* 14·6 mg/l, SEM 1·74; $P < 0.001$ respectively). All reported variables returned to their normal values 24 h after cessation of mimosine infusion except feed intake which was affected for a longer period. Mohair length and diameter were not affected by mimosine infusion. The toxicity of mimosine may be due to the drastic depletion of Zn and Mg in the blood as mimosine possesses very strong chelating properties and is excreted in the urine as a chelate.

Angora goats: Mimosine: Skin perfusion

Mimosine (β-(*N*-(3-hydroxy-4-oxopyridyl))-α-aminopropionic acid) is a naturally occurring toxic amino acid found in the seeds and leaves of *Leucaena leucocephala*. Several explanations concerning the mode of action of mimosine toxicity have been presented. Its similarity in structure to tyrosine and phenylalanine has led to the conclusion that it replaces these amino acids in proteins and enzymes (Thompson *et al.* 1969; Hegarty, 1978). Such replacement causes loss of enzyme and functional protein activity. El-Harith *et al.* (1983) have proposed a model in which the toxic effect of mimosine is explained by a deficiency of glycine for synthesis of bile acids, which in turn causes a decrease in fatty acid absorption thereby causing a deficiency of fat-soluble vitamins. A direct effect of mimosine on DNA and RNA polymerases was postulated by Reisner *et al.* (1979). Inhibition of DNA synthesis due to mimosine was observed in different cells by Ward & Harris (1976) and Hegarty *et al.* (1978). Complexing of mimosine with pyridoxal phosphate was given as a possible explanation of mimosine toxicity (Thompson *et al.* 1969; Hegarty, 1978). Lack of

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pyridoxal coenzymes causing inhibition of many enzymes linked to amino acid metabolism, such as cystathionine γ-lyase (*EC* 4.4.1.1; Unterhalt, 1980) and tyrosine decarboxylase (*EC* 4.1.1.25; Crounse *et al.* 1962), has also been reported. Another possible factor in mimosine toxicity is the inhibition of superoxidase dismutases such as succinate dehydrogenase (*EC* 1.3.5.1; Tsai, 1961) and alkaline phosphatase (*EC* 3.1.3.1; El-Harith *et al.* 1981).

Changes in wool growth and other variables have been observed in animals fed on diets high in mimosine. Szyszka & Meulen (1985) reported that there were no signs of mimosine poisoning when sheep were fed on diets containing *Leucaena* leaf (0·14 g mimosine/kg body weight (BW)); however, the animals subsequently cast their fleece. When sheep were fed on a diet containing 300 or 600 g *Leucaena*/kg (providing 0·13 or 0·26 g mimosine/kg BW per d), fleece loss was observed after 10 d of continuous feeding but no differences were noted between the two treatments (Franzolin & Velloso, 1986). Ward & Harris (1976) demonstrated inhibition of DNA synthesis *in vitro* in sheep wool follicle bulb cells. Mimosine was investigated as a potential defleecing agent in Australia. Reis (1978) observed that intravenous infusion of mimosine at the rate of 80 mg/kg BW per d in sheep for 2 d produced consistent defleecing. It could be concluded, therefore, that mimosine toxicity affects skin metabolism and wool growth very quickly and changes in wool growth are the first visible symptoms of mimosine toxicity. Thus, the possibility exists that a particular area of skin infused with mimosine would show differences when compared with an area infused with saline. If mimosine changes skin metabolism directly, changes in blood variables are also expected.

The objective of the present study was to investigate whether there was a local effect of mimosine on blood metabolites and fibre growth using a skin perfusion model developed in our laboratory.

MATERIALS AND METHODS

Animals and surgery

Four Angora wethers (body weight (BW) 35 (SE 2·3) kg) from the research herd of E. (Kika) de la Garza Institute for Goat Research were used in the present study. Animals were anaesthetized with ketamine hydrochloride (20 mg/kg BW; Ketaset, Aveco Co., Fort Dodge, IA, USA) and implanted bilaterally with silicone catheters in the superficial branches of the deep circumflex iliac artery (i.d. 0·3 mm, o.d. 0·63 mm) and in the deep circumflex iliac vein (i.d. 0·51 mm, o.d. 0·94 mm) as described by Pierzynowski *et al.* (1994). An indwelling catheter was inserted into the carotid artery. Animals recovered from anaesthesia within 5–6 h and were moved to individual cages where they were allowed to recover for 1 week. For the experimental period the animals were offered a complete mixed diet (110 g crude protein and 10 MJ metabolizable energy/kg DM). Animals were fed *ad lib.* once daily at 08.00 hours, and had free access to water.

Experimental procedures

Experimental design. The experiment was divided into five periods. On day 1 blood was collected from all three catheters to determine initial levels of metabolites (pre-infusion control period, PRC; -36 and -24 h). On day 2 saline (9 g NaCl/l; 10 ml/h) was infused bilaterally to the deep circumflex iliac artery and blood was taken twice (pre-infusion saline period, PRS; -12 and 0 h). On days 3–5 mimosine (40 mg/kg metabolic weight ($W^{0.75}$) per d) diluted in saline was infused at a rate of 10 ml/h to the deep circumflex iliac artery in the treated side. The control side received saline at the same rate of 10 ml/h. Blood was collected twice daily during the mimosine period (MI; 12, 24, 36, 48, 60 and 72 h). Following cessation of mimosine infusion on day 5 (post-infusion saline period, PSS) all goats

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continued to receive the saline bilaterally and blood samples were collected at 84 and 96 h. On days 7 and 8 of the experiment, post-infusion control (PSC) blood samples were collected (108 and 136 h). At 3 weeks after cessation of infusion a mohair sample from the perfused region was collected for analysis of fibre length and diameter. At the same time the perfused areas were checked to determine if mohair could be removed by hand.

Materials. The mimosine used was from Koa hoale seed and was 99% pure (Sigma Chemical Company, St Louis, MO, USA). The infusions were performed using microinfusion pumps (Siropump, Everest Electronic, Seaford, Australia).

Blood collection. Blood samples were collected from both treated (mimosine infusion) and control (saline infusion) catheterized deep circumflex iliac veins as well as from the carotid artery (peripheral blood). Blood was collected into 7 ml capacity tubes containing either potassium oxalate and NaF or sodium heparin or EDTA(K_3) (Becton Dickinson Vacutainer Systems, Rutherford, NJ, USA). The tubes were immediately chilled in an ice bath and transported to the laboratory where they were centrifuged at 1500 *g* at 4° for 20 min. Plasma samples were stored at −20° until analysis.

Analyses. Plasma hormones were analysed using commercially available kits from ICN Biomedicals, Inc. (Costa Mesa, CA, USA); insulin (Cat. No. 07-160102), cortisol (Cat. No. 07-221102), total triiodothyronine (T_3) (Cat. No. 07-292102) and total thyroxine (T_4) (Cat. No. 07-290102). The intra-assay CV averaged 10·3% for insulin, 7·68% for cortisol, 6·6% for T_3 and 5·9% for T_4. Amino acid analyses were performed using AminoQuant (Hewlett Packard, San Fernando, CA, USA) using precolumn derivatization with *o*-phthalaldehyde and 9-fluorenylmethylchloroformate and u.v. detection. Plasma (1 ml) with added internal standard (norvaline and sarcosine, 0·1 ml) was deproteinized with 0·9 ml Seraprep (Pickering, Mountain View, CA, USA). Plasma mimosine was estimated using a HPLC method described by Tangendjaja & Wills (1980). Plasma Zn, Mg, Fe and Cu concentrations were estimated using plasma emission spectroscopy (Applied Research Laboratories, Inc., Dearborn, MI, USA), according to the method of Seeley & Kinsey (1982). Plasma glucose concentrations were analysed colorimetrically using a Sigma glucose diagnostic kit (Cat. No. 315 Sigma Diagnostic, St Louis, MO, USA). Plasma non-esterified fatty acid (NEFA) concentration was analysed using a Wako NEFA test kit that utilizes an enzymic method for the quantification of non-esterified (or free) fatty acids (Wako Pure Chemical Industries, Osaka, Japan; Cat. No. 990-75401). Plasma creatinine was estimated using a creatinine diagnostic kit (Cat. No. 555-A, Sigma Diagnostic) and total protein by diagnostic kit (Cat. No. P6529, Sigma Diagnostic). Plasma urea N was determined as described by Sahlu *et al.* (1992). Fibre length was determined according to American Society for Testing and Materials (1988) standards and fibre diameter was determined using the Peyer FDA 200 System (Wallerau, Switzerland).

Statistical analysis

Statistical analysis of the data indicated that there was no local effect of mimosine on the skin, except for mimosine concentration. However, there was a systemic animal effect. Therefore the data were analysed for these effects using only the data from the treated side. Data were analysed by ANOVA with single degree of freedom contrasts (Statistical Analysis Systems, 1985). The model used for analysis of most variables included animal and period and the interaction of animal and period which was used as the error term. The first contrast tested for differences between the PRC and PRS periods respectively. When there were no differences between these two periods they were combined and contrasted against the MI period. To test the carry-over effects the PSS period was contrasted with the PRC and PRS periods. To test if the animals had recovered by 36 h after mimosine infusion the PSC period was contrasted with PRC and PRS periods. The data for plasma mimosine

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concentrations were analysed as a split-plot design. Mohair data were analysed as a randomized complete block design where animals were blocks and side of the animals treatment (Steel & Torrie, 1980).

RESULTS AND DISCUSSION

Mimosine infusion (from 0 to 72 h) increased plasma mimosine concentration ($P < 0.01$) in the iliac vein on the mimosine-treated side above that in both the iliac vein on the control side and the carotid artery (Fig. 1). Mimosine was cleared from the bloodstream within 12 h of cessation of mimosine infusion. Szyszka & Meulen (1984) reported that 60% of ingested mimosine was excreted in the urine within 8 h in rats, suggesting that very little mimosine metabolism occurs in the cells. Mosca *et al.* (1992) using a CHOC 400 cell line culture demonstrated that cellular uptake of mimosine is slow. They concluded that either a lag time is required to convert mimosine to an active form capable of inhibiting cell function or that mimosine is not directly involved in cell metabolism.

In the present experiment plasma mimosine concentration in the iliac vein on the mimosine-treated side (Fig. 1) was higher than that reported in peripheral blood of Merino sheep infused systemically with mimosine for 2 d (100 μmol/l; Reis, 1978). Reis (1978) observed that wool growth ceased approximately 1 d following the onset of infusion and that the sheep were then able to be manually defleeced. In the present study mimosine did not influence mohair growth even though it was infused for 3 d. Mohair length and diameter were not affected as a result of mimosine infusion (Table 1), and were similar to values for this time of the year and period of growth observed by Puchała *et al.* (1995*a*). This indicates that Angora goats respond differently to mimosine from Merino sheep. Hoey & Hopkins (1983), using sheep cannulated in the retrograde fashion to the deep circumflex iliac arteries, reported that wool was readily plucked by hand from skin preparations infused for 2 d with 0·6 g mimosine/d. These authors reported that the depilatory effect was observed only for the skin preparation area and suggested that mimosine affected the area directly.

In the present experiment mimosine infusion decreased feed and water consumption (Table 2). The goats refused feed on the third day of mimosine infusion but regained their appetite 3 d after cessation of mimosine infusion. Water consumption was similarly affected. Water consumption decreased faster than feed consumption, but at no time did water intake cease completely. Feed and water intake was lowest during the MI and PSS periods. Feed intake in sheep was also decreased by mimosine infusion (Hegarty, 1978); however, the infusion rate was four times higher than in the present experiment.

During the MI and PSS periods plasma glucose concentration increased and corresponded to an increase in plasma NEFA (Table 3). It is difficult to explain the simultaneous increase in these metabolites and why plasma glucose was elevated in goats with depressed feed intake. Plasma NEFA levels remained elevated during the PSC period. Plasma T_3 and T_4 concentrations declined during MI (Table 4). These results are in agreement with the work of Jones & Hegarty (1984), but in contrast to the reports of Jacquement *et al.* (1990) and Fernandez *et al.* (1991). Plasma amino acid concentrations decreased during MI with the exception of phenylalanine. In most cases the decrease was twofold or greater (Table 5; Fig. 2). Although animals refused to eat only on the third day of mimosine infusion, changes in blood metabolites were observed much earlier (Table 5; Figs. 2 and 3). Following cessation of mimosine infusion, concentrations of plasma amino acids returned to pre-infusion levels much faster (PSS period) than feed intake (PSC period). Changes in amino acid concentrations cannot be totally attributed to reduced feed intake since they returned to pre-infusion concentrations before resumption of feed intake.

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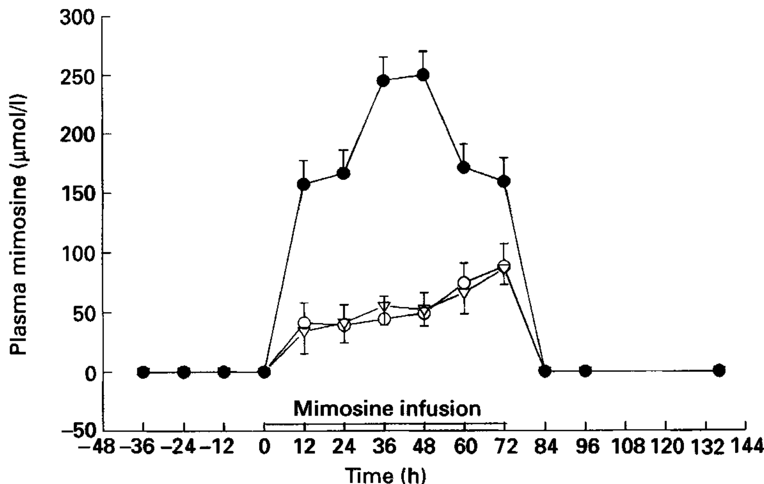

Fig. 1. Plasma mimosine concentration (µmol/l) during mimosine infusion (40 mg/kg $W^{0.75}$ per d) to the superficial branch of the deep circumflex iliac artery of Angora goats. Values are means for four goats, with their standard errors indicated by vertical bars. Values for the treated side (●) differed significantly from those of the control side (○) and carotid artery (▽) throughout the mimosine infusion ($P < 0.01$).

Table 1. *Mohair measurements from skin preparations in Angora goats after 21 d cessation of mimosine infusion**

(Mean values for four goats, and the pooled standard error of the mean)

Item	Infusion: Mimosine	Infusion: Saline	SEM	Statistical significance
Mohair				
Length (mm)	16·0	16·0	0·36	NS
Diameter (µm)	28·7	29·0	0·80	NS

* Total area of the skin supplied by the superficial branch of the deep circumflex iliac artery was about 300 cm², an area of 50 cm² located in the middle of perfused region was used for mohair measurements. Mimosine (40 mg/kg $W^{0.75}$ per d) was infused intra-arterially into one iliac artery of each goat for 3 d and saline was infused in the contralateral (control) iliac artery.

Table 2. *Feed and water intake by Angora goats during mimosine infusion**

(Mean values for four goats with the pooled standard error of the mean)

	Pre			Post			Contrast			
Item	PRC	PRS	MI	PSS	PSC	SEM	PRC v. PRS	PRC +PRS v. MI	PRC +PRS v. PSS	PRC +PRS v. PSC
Feed (kg/d)	2·3	2·3	1·1	0·6	1·8	0·29	NS	0·001	0·001	NS
Water (litres/d)	5·0	5·2	2·1	1·3	3·7	0·67	NS	0·001	0·001	NS

PRC, pre-infusion control (−36, −24 h); PRS, pre-infusion saline (−12, 0 h); MI, mimosine infusion (12–72 h); PSS, post-infusion saline (84–96 h); PSC, post-infusion control (108, 136 h).

* Infusion of mimosine (40 mg/kg $W^{0.75}$ per d) to the superficial branch of the deep circumflex iliac artery was started at 0 h and ceased at 72 h.

Table 3. *Concentration of plasma metabolites in Angora goats during mimosine infusion**†

(Mean values for four goats with the pooled standard error of the mean)

	Pre			Post			Contrast			
Item	PRC	PRS	MI	PSS	PSC	SEM	PRC *v.* PRS	PRC +PRS *v.* MI	PRC +PRS *v.* PSS	PRC +PRS *v.* PSC
Glucose (mmol/l)	3·21	3·40	4·05	4·28	3·52	0·43	NS	0·026	0·01	NS
NEFA (mmol/l)	226·4	247·2	421·8	368·5	367·8	35·88	NS	0·001	0·001	0·001
Creatinine (mg/l)	9·1	6·4	6·2	5·2	4·2	0·39	0·001	0·02	0·02	0·02

PRC, pre-infusion control (−36, −24 h); PRS, pre-infusion saline (−12, 0 h); MI, mimosine infusion (12–72 h); PSS, post-infusion saline (84–96 h); PSC, post-infusion control (108, 136 h); NEFA, non-esterified fatty acids.

* Infusion of mimosine (40 mg/kg $W^{0.75}$ per d) to the superficial branch of the deep circumflex iliac artery was started at 0 h and ceased at 72 h.

† Due to lack of a local response to mimosine only data from the treated side were used.

Table 4. *Concentrations of hormones in the plasma of Angora goats during mimosine infusion**†

(Mean values for four goats with the pooled standard error of the mean)

Item	PRS	MI	PSC	SEM	PRS *v.* MI	PRS *v.* PSC
Insulin (μIU/ml)	28·7	17·3	35·9	1·89	0·01	NS
Cortisol (μg/l)	14	62	15	3·5	0·001	NS
Triiodothyronine (ng/l)	1495	695	1196	43·1	0·001	0·01
Thyroxine (μg/l)	61·5	19·5	43	1·8	0·001	NS

PRS, pre-infusion saline (−12, 0 h); MI, mimosine infusion (12–72 h); PSC, post-infusion control (108, 136 h).

* Infusion of mimosine (40 mg/kg $W^{0.75}$ per d) to the superficial branch of the deep circumflex iliac artery was started at 0 h and ceased at 72 h.

† Due to lack of a local response to mimosine only data from the treated side were used.

Similar patterns were also observed for plasma mineral concentrations (Table 6, Fig. 3). Plasma glucose level increased during MI and PSS periods contrary to that observed in response to starvation or reduced feed intake (Hayden *et al.* 1993; Norton *et al.* 1993). It is not known why plasma creatinine concentrations (Table 3) were higher before mimosine infusion (PRC period); however, there were no observed changes in plasma creatinine during or after mimosine infusion. In animals undergoing starvation plasma creatinine has been shown to be correlated with the nutritional status (energy and protein) of the animal (Muhlbauer & Oswald, 1992). It was suspected that mimosine may elevate plasma metabolites as a result of decreased water intake and increased urinary excretion. Previous studies in our laboratory have shown that packed cell volume increases as a result of infusing mimosine or feeding *Leucaena* (T. Sahlu, S. G. Pierzynowski and R. Puchała, unpublished results).

In the present study plasma concentrations of Zn and Mg decreased during mimosine infusion (Table 6; Fig. 3). Plasma concentrations of Mg and Zn are reliable indicators of Mg and Zn deficiencies (Lamand *et al.* 1983). The decrease in plasma Zn was similar to that

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Table 5. *Concentration (μmol/l) of plasma amino acids of Angora goats during mimosine infusion**†

(Mean values for four goats with the pooled standard error of the mean)

	Pre			Post			Contrast			
Item	PRC	PRS	MI	PSS	PSC	SEM	PRC *v.* PRS	PRC +PRS *v.* MI	PRC +PRS *v.* PSS	PRC +PRS *v.* PSC
Glu	43·2	44·2	27·0	46·9	64·9	2·48	NS	0·001	NS	0·02
Ser	150·7	140·1	50·8	92·5	104·5	5·30	NS	0·001	0·02	0·02
Gly	810·3	785·5	364·6	858·6	785·5	35·33	NS	0·001	NS	NS
Ala	197·9	178·4	113·4	197·9	212·2	8·77	NS	0·001	NS	NS
Arg	147·2	154·8	68·8	163·4	127·3	14·6	NS	0·001	NS	0·01
Tyr	73·2	70·4	54·8	64·5	67·7	4·87	NS	0·01	NS	NS
Val	257·7	253·7	97·7	180·6	207·2	8·48	NS	0·001	0·01	0·01
Phe	53·2	56·1	51·2	56·4	50·7	1·63	NS	NS	NS	NS
Ile	87·9	91·4	31·1	59·1	71·0	3·91	NS	0·001	0·01	NS
Leu	216·3	198·3	74·4	128·7	123·7	8·86	NS	0·001	0·02	0·02
Lys	95·9	93·1	37·4	72·9	94·8	4·28	NS	0·001	NS	NS
Met	22·7	21·7	13·1	20·3	22·1	1·41	NS	0·001	NS	NS

PRC, pre-infusion control (−36, −24 h); PRS, pre-infusion saline (−12, 0 h); MI, mimosine infusion (12–72 h); PSS, post-infusion saline (84–96 h); PSC, post-infusion control (108, 136 h).

* Infusion of mimosine (40 mg/kg $W^{0.75}$ per d) to the superficial branch of the deep circumflex iliac artery was started at 0 h and ceased at 72 h.

† Due to lack of a local response to mimosine only data from the treated side were used.

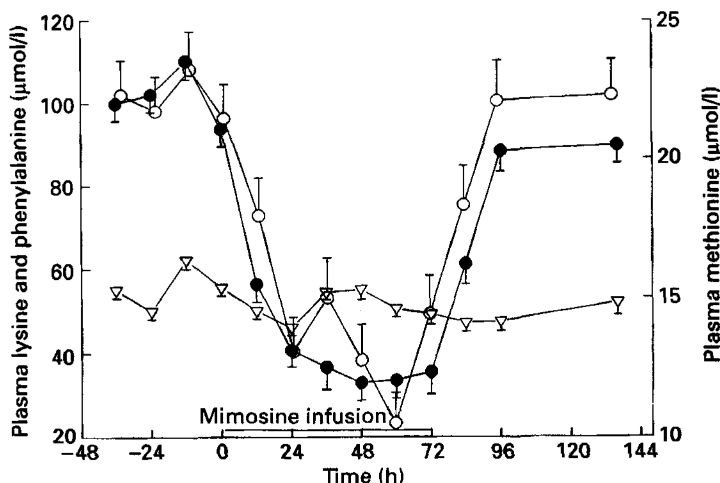

Fig. 2. Plasma lysine (●), phenylalanine (▽) and methionine (○) concentrations (μmol/l) during mimosine infusion (40 mg/kg $W^{0.75}$ per d) to the superficial branch of the deep circumflex iliac artery in Angora goats. Values are means for four goats, with their standard errors indicated by vertical bars.

observed when ewes were fed on diets containing only 10% of their Zn requirement (Masters & Moir, 1983). However, in the present experiment the time required to decrease plasma Zn was much shorter (2 d *v.* 10 weeks). It should also be noted that the average daily Zn intake in our experiment was 71 mg/d compared with 3·5 mg/d in the work of Masters

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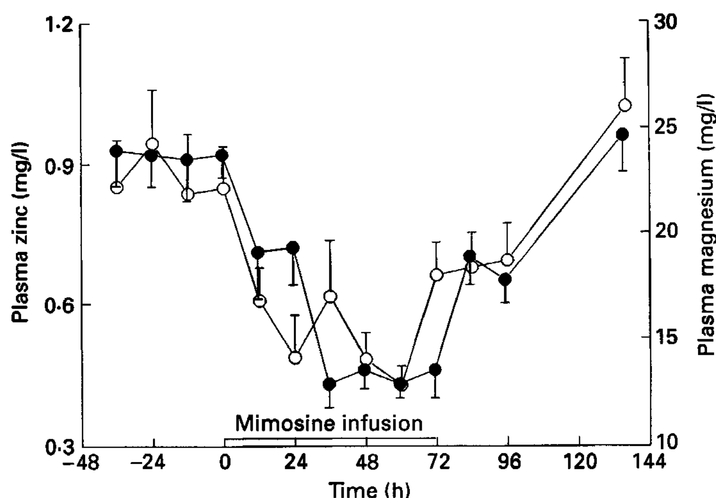

Fig. 3. Plasma zinc (●) and magnesium (○) concentrations (mg/l) during mimosine infusion (40 mg/kg $W^{0.75}$ per d) to the superficial branch of the deep circumflex iliac artery in Angora goats. Values are means for four goats, with their standard errors indicated by vertical bars.

Table 6. *Concentrations (mg/l) of plasma minerals in Angora goats during mimosine infusion**†

(Mean values for four goats with the pooled standard error of the mean)

	Pre			Post			Contrast			
Item	PRC	PRS	MI	PSS	PSC	SEM	PRC *v.* PRS	PRC +PRS *v.* MI	PRC +PRS *v.* PSS	PRC +PRS *v.* PSC
Zinc	0·97	0·91	0·49	0·67	0·95	0·063	NS	0·001	0·01	NS
Magnesium	21·4	22·1	14·6	23·9	23·0	1·74	NS	0·01	NS	NS
Iron	2·28	2·40	2·27	2·94	2·66	0·670	NS	NS	NS	NS
Copper	2·51	2·64	2·24	2·64	2·93	0·259	NS	NS	NS	NS

PRC, pre-infusion control (−36, −24 h); PRS, pre-infusion saline (−12, 0 h); MI, mimosine infusion (12–72 h); PSS, post-infusion saline (84–96 h); PSC, post-infusion control (108, 136 h).

* Infusion of mimosine (40 mg/kg $W^{0.75}$ per d) to the superficial branch of the deep circumflex iliac artery was started at 0 h and ceased at 72 h.

† Due to lack of a local response to mimosine only data from the treated side were used.

& Moir (1983). Therefore it is most likely that changes in plasma Zn were caused by mimosine infusion. Furthermore, lack of changes in plasma Cu and Fe concentrations support the concept that mimosine was responsible for the changes in plasma Zn and Mg (Tables 2 and 6; Fig. 3).

It is very difficult to relate these occurrences to the clinical symptoms of mineral deficiencies. Changes in plasma mineral concentrations occurring during mimosine infusion would not be expected to be comparable to changes caused by dietary deficiency. The decreases in plasma mineral concentrations and their return to original values were sudden in this experiment. Therefore reduced mineral concentrations may have altered several enzyme systems (Couinaud, 1984) which would not necessarily be manifested as

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clinical mineral deficiency symptoms. The only observed symptom that could be related to Zn deficiency was increased salivation.

The chelating property of mimosine is the basis for its use as a drug for treating Fe-overloading in humans (McLaren *et al.* 1983; Forsbeck *et al.* 1987; Brock *et al.* 1988). Mimosine, also, is thought to form Zn, Mg and Cu complexes (Schmid, 1988; Kontoghiorghes, 1990). In the presence of mimosine these metal ions are excreted, chelated with mimosine, via the kidney in proportion to the dose of mimosine (Schmid, 1988). The studies of Watson *et al.* (1991) and Mosca *et al.* (1992) indirectly support the hypothesis that the primary effect of mimosine is depletion of metabolic trace elements in the body. These authors demonstrated reversible inhibition of cell cycle progression by the addition of mimosine to mammalian cell cultures. Mosca *et al.* (1992) concluded that cell arrest was due to inhibition of DNA replication and that mimosine inhibits DNA synthesis possibly by inhibiting metal-dependent enzymes involved in DNA replication.

Some nutritional studies suggest that mimosine can chelate metals or can cause mineral deficiencies. The mimosine content of *Leucaena* meal was reported to be reduced by treating it with a solution of $FeSO_4$ (12·6 g/kg *Leucaena*) (Sunaria & Vidya-Sagar, 1989). Prasad (1989) observed that goats became anorexic, weak, developed frothing and erosive lesions in the mouth and had rough coats when given a ration containing 50–75% *Leucaena*. Clinical improvement, without a change of diet, was achieved by giving 2·0 g of a mineral mixture (equal parts of Zn, Cu and Fe)/d.

Urinary excretion of mimosine was not measured in the present study since that was not the objective of the experiment and it was expected that mimosine would cause changes in skin metabolism and mohair measurements. However, in another study (Puchala *et al.* 1995*b*) urinary excretion of the mimosine metabolite 2,3-dihydroxypyridine (2,3-DHP) was much faster when goats received 2,3-DHP with a mineral mixture (Zn, Mg, Fe) intraruminally compared with 2,3-DHP alone. Approximately 67% of intraruminally injected DHP was excreted during the first 24 h by the group administered DHP plus minerals compared with 30% by the group without the minerals. DHP toxicity was only observed in the group that received DHP alone. It could, therefore, be concluded that providing minerals to chelate 2,3-DHP increased the rate of toxin removal from the body.

Decreased feed intake or decreased plasma Zn concentration may have contributed, also, to changes in plasma hormones. A decrease in Zn concentration occurred during mimosine infusion (Fig. 3) and corresponded with a decrease in insulin and an increase in cortisol (Table 4). Insulin is associated with Zn in the secretory vesicles of the pancreas (Roth & Kirchgessner, 1981). A decrease in plasma Zn concentration may affect this association and thus alter insulin storage and release. Jhala & Baly (1991) using an isolated perfused pancreas technique showed that Zn deficiency decreased insulin secretion. The increase in plasma cortisol may also be due to a decrease in plasma Zn concentration. Depasquale-Jardieu & Fraker (1979) demonstrated that Zn deficiency increased the plasma cortisol level.

There was no local effect of mimosine as infused mimosine did not produce changes in skin preparations. Animals did not lose mohair in the perfused area or any other region of the body; however, changes in metabolism related to mimosine or *Leucaena* toxicity were observed. It is hypothesized that hair loss in sheep observed by others (Reis, 1978; Hoey & Hopkins, 1983) was due to the different characteristics of mohair and wool follicles (Reis & Sahlu, 1994). Decreased plasma Zn and Mg concentrations appear to be the primary mechanism of mimosine toxicity. It is suggested that the chelating property of mimosine is responsible for decreased plasma Zn and Mg concentrations. Chelating with metals may be essential for effective toxin removal by the kidney (Schmid, 1988).

https://doi.org/10.1079/BJN19960111 Published online by Cambridge University Press

This work was supported by United States Department of Agriculture Grant No. 91-38814-6240 and PCE-5053-G-00-3066-00. The authors wish to thank the farm crew at the E. (Kika) de la Garza Institute for Goat Research for animal care.

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Printed in Great Britain

https://doi.org/10.1079/BJN19960111 Published online by Cambridge University Press