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J Anim Sci 1999. 77:1224-1229.

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Effects of Mimosine and 2,3-Dihydroxypyridine on Fiber Shedding in Angora Goats¹

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ABSTRACT: The effects of intravenous infusion of mimosine or 2,3-dihydroxypyridine (2,3-DHP) and the effects of oral dose level of mimosine on fiber shedding in Angora goats were determined. In one experiment, 20 mature Angora wethers (36 ± 1.9 kg BW) were infused for 2 d with 79, 102, or 135 mg/(kg BW·d) of mimosine, 90 mg/(kg BW·d) of 2,3-DHP, or saline. At 7 d after infusion began, fiber shedding was observed in all goats receiving mimosine but not in any goats infused with 2,3-DHP or saline. Fiber shedding varied among goats; in some goats, fiber shedding was complete and occurred without hand-plucking, whereas in others fiber was retained by nonshed fibers but could be removed by hand-plucking. Nonshed fibers were larger in diameter and more likely to be medullated ($P < .05$) compared with hand-plucked fibers. Mean plasma mimosine concentration at 24 and 48 h after infusion began was 79 and 98 $\mu\text{mol/L}$ ($P < .05$), respectively, and greater ($P < .05$) for mimosine infused at 135 than at 102 mg/(kg BW·d) (89, 68, and 108 $\mu\text{mol/L}$ for mimosine infused

at 79, 102, and 135 mg/[kg BW·d], respectively; SE 9.5). In another experiment, oral dosing of eight Angora bucks ($23 \pm .5$ kg BW) with 400 or 600 mg/kg BW of mimosine rapidly increased plasma mimosine concentration, which reached approximately 100 and 160 $\mu\text{mol/L}$ at 5 h after dosing; however, periods of time during which plasma mimosine concentrations were comparable to those in the first experiment were considerably shorter. Oral mimosine dosing did not induce fiber shedding in 7 d. After 31 d, fiber was retained by nonshed fibers but could be removed by hand-plucking or could only be partially removed with difficulty by hand-plucking. There were no toxic effects of mimosine or 2,3-DHP administration; only minor, short-term inhibitions of feed intake by mimosine were noted in some goats. In conclusion, mimosine holds promise as a safe means to remove fiber of Angora goats; further research is necessary to characterize the seasonality of follicle activity and to develop convenient means of mimosine delivery.

Key Words: Angora Goats, Mimosine, Fleece, Mohair

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J. Anim. Sci. 1999. 77:1224–1229

Introduction

Leucaena (*Leucaena leucocephala*) is used as a livestock forage in tropical and subtropical regions. However, seeds and leaves contain mimosine, a toxic, nonprotein amino acid-like compound that causes alopecia in various species and fiber shedding in sheep (Hegarty et al., 1964). Mimosine has been studied in Merino sheep as a potential chemical defleecing agent (Reis et al., 1975b, 1978; Reis, 1978). Mimosine is rapidly removed from the body, and i.v. infusion for 2

d at 80 to 100 mg/(kg BW·d) caused fiber shedding 7 to 10 d after treatment commenced (Reis et al., 1975a; Reis, 1978). These infusions yielded a plasma mimosine concentration of approximately 100 $\mu\text{mol/L}$. Sheep can be defleeced by single oral doses of 400 to 600 mg/kg BW of mimosine (Reis et al., 1978); plasma concentration was greater than 100 $\mu\text{mol/L}$ 24 h after dosing.

In the rumen, mimosine is converted to 3-hydroxy-4(1H)-pyridone (**DHP**; Hegarty et al., 1964; Reis et al., 1975b), which is not depilatory in sheep (Reis et al., 1978). Some 3,4-DHP may be converted to 2,3-DHP (Jones, 1985; Allison et al., 1990; Hammond et al., 1992), but depilatory properties of 2,3-DHP have not been examined.

Mimosine or other defleecing agents (Panaretto et al., 1989) may be useful for removing fiber from Angora goats and sheep. Thus, Jacquemet et al. (1990) infused i.v. five Angora goats with mimosine for 2 d at 75 mg/(kg BW·d). Fiber growth was

¹This research was supported by USDA Grant No. 97-38814-4150. The authors wish to thank the farm crew of the E (Kika) de la Garza Institute for Goat Research for care of the animals, and Ping Wu Lee for fiber measurements.

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Received June 15, 1998.

Accepted September 24, 1998.

unaffected in two goats, but there was partial or complete alopecia within 10 d in the other three goats. Puchala et al. (1996) did not affect mohair fiber growth or elicit defleecing with a 3-d perfusion of a local area of skin of Angora goats with mimosine at 20% of a whole animal defleecing dose for sheep (Reis, 1978). Objectives of our study were to determine efficacy for removing fiber of Angora goats by 2-d i.v. infusion of different levels of mimosine or one level of 2,3-DHP. Effects of two levels of an oral dose of mimosine were also investigated.

Materials and Methods

Preliminary Experiment

Three mature Angora wethers (33 ± 1.4 kg BW), not previously exposed to mimosine, were placed in metabolism crates in late October. Goats in this and subsequent experiments were cared for in accordance with guidelines of the Consortium (1988). A 12% CP (DM basis) diet (46% cottonseed hulls, 45% ground corn, 7% soybean meal, .5% calcium carbonate, .3% dicalcium phosphate, 1% trace-mineralized salt [94 to 95% NaCl and at least .2% Mn, .16% Fe⁺², .14% Fe⁺³, .033% Cu, .1% Zn, .007% I, and .005% Co], and .2% vitamin premix [2,200 IU/g vitamin A, 2,200 IU/g vitamin D₃, and .2 IU/g vitamin E]) was fed once daily (1400) at 40 g/kg^{.75} BW. After 2 d to adjust to experimental conditions, goats were infused for 38 or 39 h with 81 ± 3.0 mg/(kg BW·d) of mimosine. Mimosine (β [N-(3-hydroxy-4-oxopyridyl)]- α -aminopropionic acid) was isolated from seeds of *Leucaena leucocephala*, and mimosine infusion solutions were prepared as described by Reis et al. (1975a). Solutions (daily volume of 400 to 500 mL) were infused continuously using a miniperistaltic pump (Harvard Apparatus, South Natick, MA) and a jugular vein catheter.

From 7 to 35 d after the start of the infusion, goats were inspected daily for evidence of fiber shedding and to determine whether fiber could be readily removed by hand-pulling. These data were observational in nature and not statistically analyzed.

Infusion Experiment

Twenty mature Angora wethers (36 ± 1.9 kg BW) were placed in metabolism crates a few days after the infusion phase of the preliminary experiment, and most procedures were the same as in the preliminary experiment. There were five treatments, with four goats per treatment, and infusions lasted approximately 48 h. The control treatment consisted of infusion of .9% (wt/vol) saline. Mimosine was infused at $79.1 \pm .81$, $101.6 \pm .57$, and 134.7 ± 1.41 mg/(kg BW·d). The 2,3-DHP (Aldrich Chemical Co., Milwaukee, WI) was infused at $89.7 \pm .61$ mg/(kg BW·d),

which is the molar equivalent of 160 mg/(kg BW·d) of mimosine. Preparation of the 2,3-DHP solution was similar to that of mimosine solutions, except that to maintain solubility it was necessary to adjust pH to between 9.0 and 9.5. In addition, blood samples at 24 and 48 h after infusion began were collected via jugular venipuncture into vacuum tubes containing sodium heparin (Becton Dickinson, Rutherford, NJ). Immediately after collection, blood was chilled in an ice bath and centrifuged at $1,500 \times g$ at 4°C for 20 min. Plasma was stored at -20°C until mimosine and 2,3-DHP analyses (Tangendjaja and Wills, 1980).

Fiber shedding occurred in all goats treated with mimosine and not in goats infused with saline or 2,3-DHP. Three types of fiber samples from mimosine-treated goats were collected from an area on the midside at 8 or 9 d after infusion began. Fiber that could be shed was obtained by hand-plucking. All fiber present was obtained by clipping (Oster size 40 blade), which included fiber that could be hand-plucked and nonshed fiber. Nonshed fibers were obtained by clipping fibers not removed by hand-plucking. However, fibers from the midside of two goats had already been shed at the time of sampling; hence, fiber on a thigh was sampled. Also, one goat had no nonshed fibers at the time of sampling. Mean fiber diameter and the percentage of medullated fibers were measured on samples of 400 snippets using an optical fiber diameter analyzer (OFDA 100; Zellweger Uster, Charlotte, NC), with previous validation for wool and mohair (Qi et al., 1994).

Data were analyzed by the GLM procedure of SAS (1990). Fiber diameter and percentage of medullated fibers were analyzed as a split plot, with a main plot of mimosine infusion level and a subplot of fiber type (e.g., clipped, plucked, and nonshed). Plasma concentration of mimosine in goats infused with mimosine was analyzed as a split plot in time. Because 2,3-DHP was detectable only in goats infused with 2,3-DHP, its concentration for these animals was analyzed with day in the model. Means for individual treatment combinations are presented, and main effect means are listed as well when significant ($P < .05$) differences existed. Differences among means were determined by least significant difference with a protected *F*-test.

Oral Dose Experiment

A few days after the infusion phase of the infusion experiment, eight Angora bucks ($23 \pm .52$ kg BW) were used in an experiment with most procedures similar to those of the infusion experiment. The two treatments entailed oral dosing of 400 or 600 mg/kg BW of mimosine, which was suspended in approximately 400 mL of water and delivered to the reticulorumen via a stomach tube. Blood samples were collected as described earlier at 3, 5, 22, 28, and 46 h after dosing. Plasma concentration of mimosine was

statistically analyzed as a split plot in time, with differences among means determined by least significant difference and a protected *F*-test.

Results

Preliminary Experiment

Mimosine infusion for 38 or 39 h induced shedding in two of the three goats. However, shedding was incomplete, even 5 wk after infusion. These observations dictated testing of higher mimosine infusion levels in the infusion experiment.

Infusion Experiment

Fiber was shed by all goats receiving mimosine at 7 d after infusion began but not by any goats given saline or 2,3-DHP. The nature of shedding was quite variable among animals; fiber of two mimosine goats was shed without hand-plucking and was not retained

by nonshed fibers at 7 d, whereas, for other goats, fiber was retained to variable extents by nonshed fibers. However, these fleeces could be easily removed by hand-plucking (Figure 1a, b, and c). By 3 wk after infusion, some fleeces retained by nonshed fibers at 7 d had been completely shed without hand-plucking, although for other goats a small number of nonshed fibers remained after hand-plucking. These differences were not related to mimosine level. Fiber regrowth for all goats that received mimosine was apparent by 3 wk after infusion (Figure 1d).

Interactions in diameter and percentage of medullated fibers between mimosine infusion level and fiber type were nonsignificant ($P > .05$; Table 1). For goats infused with mimosine, fibers that could be removed by hand-plucking were similar ($P > .05$) to those clipped in diameter and percentage medullated fibers. In contrast, nonshed fibers were largest among fiber sample types in diameter and greatest in concentration of medullated fibers ($P < .05$). Infusion level did not affect fiber diameter or percentage of medullated fibers ($P > .05$).



Figure 1. Fleece removal and regrowth after intravenous infusion of mimosine for 2 d (infusion experiment). (a) and (b): Goats partially defleeced by hand-pulling 7 d after the start of infusion of 135 mg/(kg BW·d) of mimosine. (c): Goat partially defleeced by hand-plucking 10 d after the start of infusion of 79 mg/(kg BW·d) of mimosine. (d): Fiber regrowth 23 d after the start of infusion of 135 mg/(kg BW·d) of mimosine.

Table 1. Fiber characteristics for Angora goats infused intravenously with mimosine for 2 d (infusion experiment)

Item	Fiber type	Mimosine infusion rate, mg/(kg BW·d)			SE	Mean
		79	102	135		
Diameter, μm	Clipped	29.4	29.6	28.7	1.36	29.2 ^a
	Plucked	29.5	29.5	28.2		29.1 ^a
	Nonshed	31.7	32.7	32.3 ^c		32.3 ^b
SE						.79
Medullated, %	Clipped	2.4	2.2	2.3	4.63	2.3 ^a
	Plucked	1.8	1.9	1.7		1.8 ^a
	Nonshed	11.4	22.9	19.1 ^c		17.9 ^b
SE						2.68

^{a,b}Within an item and column, means lacking a common superscript letter differ ($P < .05$).

^cThree observations per mean; four observations per mean for others.

For plasma mimosine concentration, there was no interaction between mimosine infusion level and time of sampling ($P > .10$; Table 2). The plasma concentration of mimosine was greater ($P < .05$) at 48 than at 24 h after mimosine infusion began. Plasma mimosine concentration was greater ($P < .05$) for infusion at 135 than at 102 mg/(kg BW·d) and, unexplainably, intermediate ($P > .05$) for the lowest rate. No 2,3-DHP was detected in plasma of goats infused with mimosine.

Infusion with 2,3-DHP did not elicit detectable mimosine concentration in plasma, as expected. Concentration of 2,3-DHP in plasma of goats infused with 2,3-DHP was similar ($P = .30$) at 24 and 48 h after infusion began (33.5 and 64.6 $\mu\text{mol/L}$, respectively; SE 19.39).

No toxic effects of mimosine or 2,3-DHP infusion were noted. Most goats continued to consume feed normally during the infusion period. However, one goat subjected to the highest level of infused mimosine did not consume feed on d 1, and, for two other goats (one for the low and one for the moderate rate of mimosine infusion), there were small feed refusals (i.e., 10 to 20% of that offered) on one or both infusion days.

Oral Dose Experiment

At 7 and 9 d after oral dosing of mimosine, no fiber shedding was noticeable. However, at 31 d, changes in fleece status of some goats were observed. For two goats dosed with 400 mg/kg BW of mimosine, the fibers were largely retained by nonshed fibers but could be hand-plucked. For another goat on this treatment, fiber could be removed with difficulty by hand-plucking. No changes occurred in fleece status of the fourth goat on this treatment. Likewise, with the 600 mg/kg BW of mimosine dose, fiber of two goats retained by nonshed fibers could be removed by hand-plucking, fiber of one goat exhibited weakness but

could not be defleeced, and there was no effect with the fourth goat.

Level of mimosine orally dosed and time interacted ($P = .06$) for plasma mimosine concentration (Figure 2). Oral doses of mimosine caused rapid increases in plasma mimosine concentration, which reached approximately 100 and 160 $\mu\text{mol/L}$ at 5 h after dosing 400 and 600 mg/kg BW of mimosine, respectively. However, the length of time that plasma mimosine concentration was comparable to that in the infusion experiment was considerably shorter. Rates of decline in mimosine concentration, without considering the concentration at 3 h, were 2.43 ($r^2 = .89$) and 3.52 $\mu\text{mol}/(\text{L}\cdot\text{h})$ ($r^2 = .92$) for doses of 400 and 600 mg/kg BW of mimosine, respectively (SE = .510; $P = .18$). These rates may explain greater plasma mimosine concentration at 48 vs 24 h in the infusion experiment. No 2,3-DHP was detected in plasma. No toxic effects of oral dosing of mimosine were observed. However, two goats orally dosed with 600 mg/kg BW of mimosine consumed only approximately 50% of offered feed for 2 d after dosing.

Discussion

Mimosine Infusion

Defleecing. Results of this study show that mimosine is a depilatory compound in Angora goats as in sheep (Reis and Panaretto, 1979; Panaretto et al., 1989). Effects of mimosine on fiber growth are due mainly to antimitotic properties (Reis, 1979), with inhibition of DNA synthesis and, hence, decreased cell division in wool follicle bulb cells (Ward and Harris, 1976). Consequently, mimosine should only affect follicles in the anagen (i.e., active) growth phase. Merino sheep grow wool fibers continuously (Reis, 1982); therefore, mimosine treatment defleeces completely any time of the year. Conversely, Angora goats exhibit seasonality in rate of fiber growth (Reis and

Table 2. Plasma concentrations of mimosine in Angora goats infused intravenously with mimosine for 2 d (infusion experiment)

Day ^a	Mimosine infusion rate, mg/(kg BW·d)			SE	Mean
	79	102	135		
1	82.8	58.8	94.6	5.66	78.7 ^b
2	96.0	77.3	121.2		98.2 ^c
SE					3.30
Mean	89.4 ^{bc}	68.0 ^b	107.9 ^c	9.48	

^aDay 1 and 2 represent samples taken approximately 24 and 48 h, respectively, after infusion began.

^{b,c}Within a row or column, means lacking a common superscript letter differ ($P < .05$).

Sahlu, 1994). Most secondary follicles of Angora goats in New Zealand grow continuously, whereas up to 75% of primary follicles enter a telogen (e.g., resting) phase during winter months (Nixon et al., 1991). Thus, many primary follicles in skin of Angora goats might not shed fiber at this time (December to February in the northern hemisphere) in response to mimosine or other depilatory compounds. Hence, in the present study, it was possible that some follicles were in a telogen phase. Greater fiber diameter and higher percentage of medullation in nonshed fibers probably can be explained by a higher proportion of primary fibers. If most follicles producing medullated fibers are in a telogen phase for a defined period of time, they could be separated from the remaining fleece by dosing with mimosine. Therefore, a full understanding of seasonality in follicle activity for Angora goats in the United States is necessary to most effectively use depilatory compounds such as mimosine to defleece.

Reis (1978) found that the minimal level of intravenous infusion of mimosine required to consis-

tently defleece Merino sheep was 80 mg/(kg BW·d), given for 2 d. Defleecing was associated with a concentration of mimosine in plasma approaching 100 $\mu\text{mol/L}$. Angora goats seem to be similar to Merino sheep, in that defleecing in the infusion experiment consistently occurred when mimosine was infused for 2 d at 80 mg/(kg BW·d), concomitant with a plasma mimosine concentration of 80 to 100 $\mu\text{mol/L}$. However, infusion at this level for 38 or 39 h in the preliminary experiment was marginally effective. The failure of 2,3-DHP to defleece goats in the infusion experiment reflects that, as with 3,4-DHP in sheep (Reis et al., 1978), DHP is not a depilatory compound. During the infusion of mimosine, no DHP was detected in plasma, which indicates that, as with sheep (Hegarty et al., 1964), mimosine is not degraded to DHP in animal tissues.

Toxic Effects. No adverse effects on goats of mimosine or 2,3-DHP infusion were observed, apart from minor feed refusals. With Merino sheep, toxic effects (including the death of one of four sheep) were observed when 160 mg/(kg BW·d) of mimosine was infused for 2 d (Reis, 1978). Likewise, 2,3-DHP was toxic to Angora goats when 6 g, equivalent to approximately 170 mg/kg BW of mimosine, was given as a single intraruminal dose (Puchala et al., 1995). Results of the infusion experiment suggest that 2,3-DHP infused at a rate of 90 mg/(kg BW·d) is cleared from the body rapidly enough to prevent toxic effects.

Oral Dosing of Mimosine

Single oral doses of 400 or 600 mg/kg BW of mimosine defleece most Merino sheep via a plasma mimosine concentration greater than 100 $\mu\text{mol/L}$ at 24 h after dosing (Reis et al., 1978). However, these doses in the oral dose experiment resulted in plasma mimosine concentration at 24 h lower than that noted in sheep, and the doses were only partially effective in causing fiber shedding in Angora goats. Also, periods of time during which plasma mimosine concentrations in the oral dose experiment were comparable to those in the infusion experiment were considerably shorter.

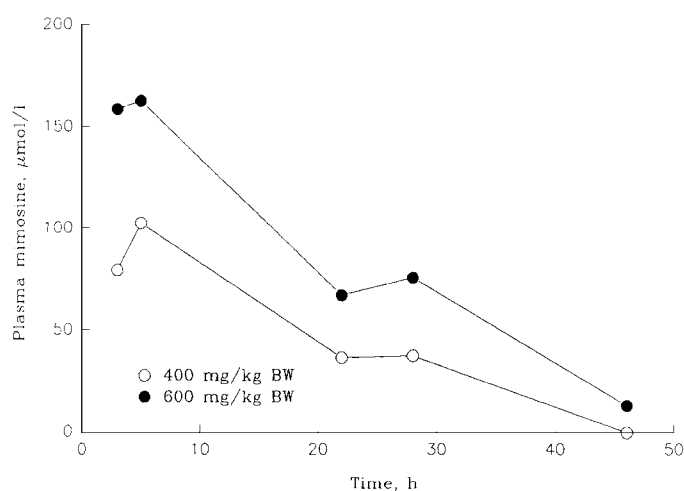


Figure 2. Plasma concentration of mimosine in Angora goats after oral dosing with 400 or 600 mg/kg BW of mimosine (oral dose experiment). SE = 13.0 $\mu\text{mol/L}$.

Implications

Mimosine is a depilatory agent for Angora goats. Two-day infusion of Angora goats with levels of mimosine similar to those effective for defleecing sheep were effective in removing Angora fiber. However, mimosine may not remove all Angora fibers, particularly primary fibers, when in a temporary resting phase. Oral mimosine administration at doses effective to defleece sheep may be less efficacious with Angora goats. Further research is required to fully characterize seasonality of follicle activity for Angora goats in the United States to most effectively use compounds such as mimosine to defleece, and to develop practical means of mimosine delivery, such as the feeding of *Leucaena leucocephala*.

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