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Effect of previous exposure of sheep to monoterpene odors on intake of alfalfa pellets treated with camphor or α-pinene

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Abstract

Lambs were subjected to odors of two monoterpenes (camphor and α-pinene) that decreased intake in a previous study to determine if exposure during feeding modified their effects on subsequent intake. In two experiments, 36 ewe lambs were group-fed alfalfa pellets in enclosed portable buildings each morning for 56 d (phase 1). Camphor (25 g, Experiment 1) or α-pinene (50 ml, Experiment 2) was placed in feeders in a mesh-covered container immediately before feeding. In phase 2, lambs were individually fed alfalfa pellets for 20 min each morning for 10 d (5-d adaptation, 5-d collection). Treatments were sprayed on alfalfa pellets at levels representing the concentration of that chemical in tarbush (*Flourensia cernua*) or at 10-fold that concentration. No day by treatment interactions were detected for intake during adaptation or collection periods for either chemical ($P > 0.05$); therefore, data were pooled across day. Exposure to the volatile aroma for 56 d had no effect on intake during the subsequent 10-d interval for either monoterpene ($P > 0.05$). Moreover, intake during the collection period was not affected by treatment concentration ($P > 0.05$). Neither concentration of the terpene applied to alfalfa pellets nor previous exposure to the volatile aroma of camphor or α-pinene altered feed intake under the conditions of this study.

Keywords: Herbivory; Intake; Monoterpenes; Olfaction; Sheep

1. Introduction

Loss of productive grasslands to shrub encroachment in arid rangelands is a serious problem for livestock producers worldwide. Livestock often avoid shrubs even though proximate analysis would suggest that they are nutritious. For example, tarbush (*Flourensia cernua* DC) is high in crude protein but contains substantial quantities of terpenes and phenolics (Estell et al., 1996), and is consumed only in limited amounts by livestock (Anderson and Holechek, 1983). Removal of surface compounds from tarbush with organic solvents increased preference by sheep (Estell et al., 1994). Specific leaf surface mono- and sesquiter-

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penes were related to tarbush consumption, with concentration of α-pinene approximately two-fold greater in low use than high use plants (Estell et al., 1998a). Monoterpenes [including camphor and (or) α-pinene] have also been shown to be related to shrub use by goats (Riddle et al., 1996) and mule deer (Personius et al., 1987). When tested individually, Vourc'h et al. (2002) reported that α-pinene was repellent to deer when applied to diets at concentrations found in western red cedar. Sinclair et al. (1988) reported that camphor was a feeding deterrent for snowshoe hares when added to their diet. Both camphor and α-pinene were negatively related to intake of sheep when applied to alfalfa pellets (Estell et al., 1998b).

Little research is available concerning the influence of volatile aromas on feeding by ruminants. Radwan and Ellis (1975) reported a greater total emission of volatile monoterpenes from Douglas fir clones that were resistant to deer browsing than from susceptible genotypes. Mayland et al. (1997) demonstrated that volatiles (primarily green leaf volatiles) released from tall fescue were related to cattle preferences. Exposure to the odor of the sulfur-containing *Astragalus bisulcatus* while eating had no persistent effect on intake of lambs (Provenza et al., 2000). Red deer rejected a pelleted diet while being exposed to the odor of five monoterpenes, including α-pinene (Elliott and Loudon, 1987). Narjisse et al. (1996) reported that sheep exposed to the aroma of a monoterpene mixture (profile similar to sagebrush) during feeding discriminated against feed associated with the terpene mixture. Our objective was to determine if exposure to camphor or α-pinene aroma during feeding affected subsequent intake by lambs when consuming a diet containing that compound. Our hypothesis was that lambs previously exposed to volatile compounds would consume more alfalfa pellets later when treated with the same chemical.

2. Materials and methods

2.1. Experiment 1

Experiments were conducted in accordance with USDA guidelines, and protocols were approved by the New Mexico State University Institutional Animal Care and Use Committee. Thirty-six Polypay ewe lambs (mean BW = 23.1 kg, approximately 3 months of age) with no previous experience browsing rangeland were adapted to alfalfa pellets (3.8% of BW, DMB) for 4 d in drylot prior to the beginning of phase 1.

2.1.1. Phase 1

Lambs were group-fed alfalfa pellets (0.64 cm o.d., 15% CP) at 3.8% of BW (DM basis) in four enclosed portable buildings (3.0 m × 3.7 m) for 2 h (08:00–10:00 h) each morning (nine lambs randomly assigned to each building) for 56 d (phase 1). Twenty-five grams of camphor were placed in feed troughs [in a screen-covered PVC tube (2.5 cm o.d., 1.35 m length) cut in half lengthwise and spanning the length of the trough] in two buildings before feeding each day (two buildings serving as controls).

2.1.2. Phase 2

After the 56-d exposure period, lambs were individually fed alfalfa pellets (640 g, DM basis) for 20 min each morning for 10 d (phase 2: d 57–66) in a metabolism unit (1.2 m × 2.4 m pens). The 20-min interval was selected because initial eating rate during a short interval at the beginning of the feeding period is a good criterion for measuring palatability of one feed and minimizes the confounding of palatability and post-ingestive effects (Baumont, 1996), and 640 g of alfalfa pellets was determined in preliminary studies to be adequate to assure some orts for each lamb after 20 min (Estell et al., 1998b). Lambs were randomly assigned to one of 12 pens and three groups, restricted such that one lamb from each building during phase 1 received each dose in each group during phase 2. The three groups ($n = 12$) were fed at 08:00, 08:30, and 09:00 h. Pellets were sprayed with camphor at one of three levels: 0× (control, ethanol carrier only), 1× (concentration on the leaf surface of tarbush), or 10× (10-fold that concentration). Orts were weighed daily, and intake was calculated for each lamb (5-d adaptation, 5-d intake measurement).

2.1.3. Animal management

Lambs were weighed weekly and feed adjusted accordingly. In addition to the treated pellets during phase 2, lambs were group-fed untreated alfalfa pellets daily at 10:00 h (mean total daily intake = 4.7% of BW, DM basis, adjusted daily to compensate for orts and weekly to account for weight gain). Lambs were maintained as one group in drylot except when feeding in phase 1 and during 20-min tests in phase 2. Alfalfa pellets were sampled randomly throughout the study, composited, ground to pass a 2-mm screen in a Wiley Mill, and analyzed for DM (94.8%; AOAC, 1990). Lambs had free access to water and trace-mineralized salt (93–97% NaCl, 3 g/kg Mn, 2.5 g/kg Zn, 1.5 g/kg Fe, 0.15 g/kg Cu, 0.09 g/kg I, 0.025 g/kg Co, and 0.01 g/kg Se) at all times.

2.2. Experiment 2

Experiment 2 was conducted exactly as Experiment 1 except that α-pinene was evaluated rather than camphor. Ewes (mean BW = 42.2 kg, approximately 7 months of age) were rerandomized to treatments, and 50 ml of α-pinene was placed in tubes in two of the four buildings daily during phase 1.

2.3. Treatments

Manufacturer (Aldrich Chemical Co., Milwaukee, WI) specified purities for camphor and α-pinene were 96 and 98%, respectively. The minimum amount of chemical necessary to maximize concentration during feeding in phase 1 was measured in preliminary tests. The amount of chemical applied during phase 2 (10× treatment, 50 and 1000 μg/g DM for camphor and α-pinene, respectively) was adjusted to account for volatility and placed in feeders in the enclosed buildings. Headspace samples were collected 10 cm from the trough center at 2 and 20 min with a gas-tight syringe and injected into a Shimadzu gas chromatograph (model GC-8A, Kyoto, Japan) equipped with a flame-ionization detector. This process was repeated with multiples (2, 4, 8, and 16-fold) of each compound. Camphor concentration, 10 cm from the tube, plateaued between the four- and eight-fold levels, while α-pinene peaked with the original quantity. Consequently, 25 g of camphor (Experiment 1) and 50 ml of α-pinene (Experiment 2) was placed in two buildings each morning before feeding during phase 1.

Stock solutions (10× dose) of camphor and α-pinene (1 and 20 mg/mL, respectively, in 100% ethanol) were diluted 10-fold in ethanol (1× dose) and applied at 0.05 mL/g of DM in phase 2. Control alfalfa pellets (0× dose) were sprayed with ethanol only. No effect of ethanol carrier on intake of alfalfa pellets by sheep was observed in a previous study (Estell et al., 2001). Treatment solutions were applied with polyethylene spray bottles in an adjacent room. Rationale for concentrations used and protocol for application to alfalfa pellets have been described previously (Estell et al., 1998b, 2000). Chemical loss due to volatilization between application and feeding was determined for each compound as described by Estell et al. (2002). Mean recovery (corrected for extraction efficiency) at 2, 10, 20, and 30 min, respectively, after application was 98.5, 95.1, 93.3, and 91.2% for camphor, and 79.3, 76.8, 69.6, and 63.4% for α-pinene. Given an approximate lag time of 10 min between application and feeding, the 10-, 20-, and 30-min estimates equate to the beginning, midpoint, and end of the 20-min feeding period.

2.4. Statistical analyses

For each experiment, intake was analyzed using a split-plot design with repeated measures in the subplot units. Buildings were whole plot units, and sheep were subplot units subjected to repeated measures. The analysis was performed using the MIXED procedure of SAS (1999). Fixed effects were pre-exposure treatment, spray treatment concentration, day, and all interactions, and random effects were building and group. A compound symmetric covariance structure was used to model the covariance structure of repeated measures after comparing it with the first-order autoregressive structure using the Baysian–Schwartz Criterion. The Kenward–Rogers option for denominator degrees of freedom was used to adjust the degrees of freedom for the split-plot structure. Adaptation period (d 1–5) and collection period (d 6–10) were analyzed separately. Adaptation periods were analyzed to examine the possibility of short-term adaptation responses.

3. Results

No day by treatment interactions were detected ($P > 0.05$) in either experiment; thus, intake data were pooled across day within adaptation and collection periods to examine main effects. Day effects were detected ($P < 0.05$) for camphor and α-pinene during the adaptation period (d 1–5), primarily due to low intake

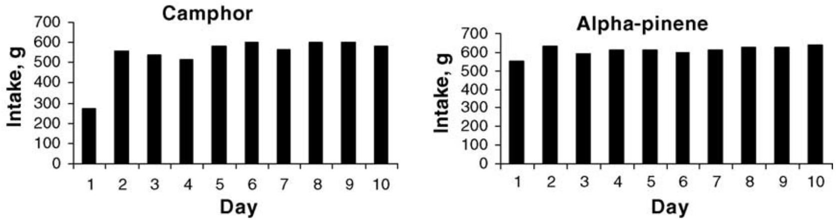

Fig. 1. Mean daily intake of lambs during a 20-min interval (phase 2: $n = 36$) in Experiment 1 (camphor) and 2 (α-pinene). A day effect was observed for camphor for d 1–5 and 6–10, and for α-pinene for d 1–5 ($P < 0.01$). S.E.M. = 21.3, 13.0, 27.8, and 32.9 for camphor d 1–5, camphor d 6–10, α-pinene d 1–5, and α-pinene d 6–10, respectively.

on d 1 in both experiments while acclimating to individual pen feeding (Fig. 1). A day effect was also detected ($P < 0.05$) for camphor during the collection period (d 6–10) in Experiment 1, with a slightly lower intake on d 7 (Fig. 1).

Pre-exposure during phase 1 had no effect on intake during phase 2 ($P > 0.05$) in either experiment (Table 1). Because no treatment effects were detected during the collection period, adaptation periods were also analyzed to examine the possibility of a rapid extinction of treatment effects. Intake during phase 2 was lower ($P < 0.05$) for lambs on the 1× concentration than on 0× or 10× for d 1–5 in Experiment 1 (camphor), and tended to be lower ($P < 0.10$) for d 6–10. No differences were detected ($P > 0.05$) among concentrations during either period for α-pinene (Experiment 2; Table 1).

4. Discussion

Our hypothesis that pre-exposure to either of these two chemicals affects subsequent intake was rejected. Although red deer (Elliott and Loudon, 1987) and sheep (Narjisse et al., 1996) discriminated against feed while exposed to the odor of α-pinene or a mixture of monoterpenes, respectively, these studies are not directly comparable because animals were exposed to terpenes during intake measurement. We are not aware of studies that pre-exposed animals to odors of volatile terpenes for comparison to our data.

The fact that intake of alfalfa pellets did not decrease linearly as concentration of either camphor or α-pinene increased contrasts with our previous findings (Estell et al., 1998b), and with deterrent effects

Table 1
Mean intake by lambs of alfalfa pellets treated with camphor or α-pinene during phase 2 after pre-exposure for 56 d[a] (g lamb^{-1} d^{-1}, DM basis)

Treatment	Camphor		α-Pinene	
	Adaptation[b]	Collection[b]	Adaptation[b]	Collection
Pre-exposure	489	588	614	624
No exposure	500	594	586	619
S.E.M.	19.0	14.7	31.8	34.4
0×	531[c]	609[e]	609	633
1×	444[d]	558[f]	613	634
10×	508[c]	605[e]	578	597
S.E.M.	23.3	18.0	29.7	35.7

[a]Lambs were exposed to aromas of camphor (Experiment 1) or α-pinene (Experiment 2) for 56 d in phase 1. Intake of alfalfa pellets treated with that compound was measured in 20-min tests for 10 d (5-d adaptation; 5-d collection) in phase 2. Concentrations of compounds applied to alfalfa pellets were multiples (0, 1, or 10×) of the concentration of that compound in tarbush; $n = 12$ and 18 for phase 1 and phase 2 treatments, respectively. [b]Day effect was detected ($P < 0.05$). [c,d]Means differed in phase 2 adaptation period for camphor ($P < 0.05$). [e,f]Means tended to differ in phase 2 collection period for camphor ($P < 0.10$).

reported for α-pinene in deer (Vourc'h et al., 2002) and camphor in hares (Sinclair et al., 1988). The reasons for these discrepancies are not clear. Lambs in the previous study (Estell et al., 1998b) were intermediate in age (6 months) and BW (35 kg) between the lambs used in the two experiments in this study. It has been suggested that plane of nutrition may account for some of the inconsistencies among studies examining the relationship of secondary compounds and diet preferences. Research indicating a relationship of terpenes and diet selection typically involved animals on a low plane of nutrition, and animals on a higher plane of nutrition may be able to ingest more aversive secondary chemicals (Illius and Jessop, 1996; Villalba et al., 2002). However, lambs in the previous study (Estell et al., 1998b) and the present study were fed at the same level (approximately 4.7% of BW, DM basis); thus, plane of nutrition cannot explain the discrepancy in this case. Because the phase 1 treatment (pre-exposure) did not affect intake, it is unlikely that acclimation to odor was responsible for the lack of effect. No evidence of post-ingestive aversion to terpenes was observed, given that the only significant day effects in either experiment were due to increased intake between d 1 and 2 (Fig. 1), and no day by treatment interactions were significant.

5. Conclusion

Neither pre-exposure to aroma of aversive terpenes (α-pinene or camphor) nor concentration of α-pinene applied affected intake of alfalfa pellets by lambs under the conditions of this study. Intake on the 1× camphor treatment was lower than for the 0 or 10× treatment, in contrast to our previous results. More work is needed to understand the complex relationship between phytochemistry and diet selection. Mechanisms to override aversive thresholds and increase intake of unpalatable shrubs by ruminants would benefit producers by increasing available forage and dietary protein [assuming minimal and (or) reversible metabolic compromise].

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