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Short communication

Effects of eugenol, α-terpineol, terpin-4-ol, and methyl eugenol on consumption of alfalfa pellets by sheep☆

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Abstract

Many secondary compounds are typically present in unpalatable shrubs on arid and semi-arid rangelands. However, the relationship between intake by livestock and concentration of individual chemicals has been examined for very few of these compounds. Four experiments were conducted to examine effects of individual volatile compounds on intake of alfalfa pellets by lambs. Forty-five lambs (9 lambs/treatment) were individually fed alfalfa pellets with eugenol, α-terpineol, terpin-4-ol, or methyl eugenol applied at one of five concentrations. Treatments were multiples (0, 0.5, 1, 2, and 10×) of the concentration (×) of each compound on the leaf surface of *Flourensia cernua*. Treatment solutions were sprayed on alfalfa pellets (0.64 kg/lamb/d, dry matter basis), and intake was measured during a 20-min interval for five days. A day effect ($P < 0.001$ for both linear and quadratic contrasts) was detected in all four experiments, but no day × treatment interactions were observed ($P > 0.05$). No treatment effects were observed ($P > 0.05$) for any of the chemicals tested; thus, eugenol, α-terpineol, terpin-4-ol, and methyl eugenol were not related to intake of alfalfa pellets by lambs under the conditions of this study.
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Keywords: Diet selection; Herbivory; Intake; Sheep; Terpenes

1. Introduction

Loss of grasslands due to shrub encroachment is a major concern for livestock producers and land managers in arid and semi-arid regions worldwide. These shrubs contain secondary chemicals and are often avoided by livestock even though they are high in protein. Secondary compounds affect mammalian diet selection through both pre- and post-ingestive processes (Provenza, 1995; Pass and Foley, 2000). Pre-ingestive chemical cues affect herbivore feeding behavior through both smell and taste (Provenza, 1995; Narjisse et al., 1996).

Sheep and goats browsing tarbush (*Flourensia cernua* DC) in small paddocks exhibited differential selectivity among plants that was related to concentration of several individual leaf surface mono- and sesquiterpenes (Estell et al., 1998a). In an effort to determine the role of volatile chemicals in feeding behavior of browsing ruminants, we conducted experiments using tarbush as a shrub model to systematically assess effects of individual leaf surface compounds on intake of alfalfa pellets by sheep. In those studies, 19 volatile compounds were

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examined (camphor, borneol, limonene, *cis*-jasmone, α-pinene, β-caryophyllene, *p*-cymene, α-humulene, 1,8-cineole, 3-carene, sabinene, camphene, myrcene, β-pinene, caryophyllene oxide, γ-terpinene, terpinolene, α-copaene, and α-terpinene; Estell et al., 1998b, 2000, 2002, 2005). When tested individually, only camphor, α-pinene, camphene, and caryophyllene oxide were related to intake. The objective of the present experiments was to continue these efforts with four previously untested volatile compounds. The hypothesis was that intake of alfalfa pellets would decrease as concentration of these compounds increased.

2. Materials and methods

Four experiments were conducted to examine effects of two oxygenated monoterpenes (α-terpineol and terpin-4-ol) and two phenylpropenes (eugenol and methyl eugenol) on alfalfa pellet intake by lambs. Mean concentrations of these compounds on the leaf surface of tarbush in previous studies (Tellez et al., 1997; Estell, unpublished data) were approximately 5, 10, 30, and 5 μg/g of dry matter (DM) for eugenol, α-terpineol, terpin-4-ol, and methyl eugenol, respectively (i.e., concentrations to which animals would be exposed when browsing tarbush). In each experiment, one compound was examined at five concentrations (treatments), with treatments being multiples (0, 0.5, 1, 2, or 10×) of the exposure concentration.

Experiments were conducted according to USDA guidelines and approved by the New Mexico State University Institutional Animal Care and Use Committee. Forty-five Rambouillet ewe lambs (approximately 12 months of age, with a mean initial body weight of 57.3 ± 1.9 kg) without previous browsing experience were used. Lambs were randomly assigned to one of 15 pens and three groups (constant across experiments). Before each experiment, lambs were randomly assigned to one of five treatments (9 lambs per treatment, restricted to 3 lambs/treatment/group) using a randomized complete block design.

Lambs were individually fed treated alfalfa pellets each morning in an enclosed metabolism unit (1.22 m × 2.44 m pens) for 20 min. Initial eating rate during a short interval at the beginning of the feeding period is a good criterion to measure palatability (Baumont, 1996). Groups (blocks) were fed at 08:00, 08:30, and 09:00 h, respectively. Each experiment lasted five days, with two preliminary 5-day adaptation periods to 1) familiarize lambs with handling and pen feeding and 2) determine baseline intake of untreated alfalfa pellets during the 20-min interval. Lambs were adapted to alfalfa pellets for five days in a drylot pen before the adaptation periods. Eugenol, α-terpineol, terpin-4-ol, and methyl eugenol were tested in experiment 1 through 4, respectively (order selected randomly, 2-day interval between experiments).

Alfalfa pellets (0.64 kg; DM basis; ≥15% CP; 0.95 cm diameter) were fed during the 20-min interval and orts were recorded daily. Alfalfa pellet samples were composited across experiments, ground in a Wiley Mill (2-mm screen), and analyzed for DM (100 °C for 24 h, DM = 92.6%). Lambs were weighed before the 08:00 h feeding on day 5 each week. Lambs were maintained as one group with free access to water and trace-mineral salt (93 to 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) except during 20-min feeding periods. At 10:00 h, lambs were group-fed untreated alfalfa pellets at 3.7% of body weight (DM basis, adjusted weekly). On test days, amount of untreated pellets fed at 10:00 h was decreased by the total amount of treated pellets eaten during the 20-min period.

All compounds were purchased from Aldrich (Milwaukee, WI) except terpin-4-ol (Acros Organics; Morris Plains, NJ). Manufacturer specified purities were 98, 90, 97, and 98% for eugenol, α-terpineol, terpin-4-ol, and methyl eugenol, respectively. Stock solutions containing 1, 2, 6, or 1 mg/ml of the respective compound in 100% ethanol were diluted 5-, 10-, and 20-fold in ethanol, corresponding to the 10×, 2×, 1×, and 0.5× treatments, respectively, when applied to alfalfa pellets at 0.05 ml/g of DM. Control pellets (0× treatment) were sprayed with ethanol. Treatments were applied using polyethylene spray bottles in an adjacent room. Treatment application protocol as well as rationale for other aspects of the design and conduct of experiments (e.g., amount of feed offered, use of alfalfa pellets as treatment carrier, etc.) were based on pilot studies that have been described in detail previously (Estell et al., 1998b, 2000).

Volatile loss between application and feeding was estimated using GC/MS as described by Estell et al. (2002). Mean recovery (corrected for extraction efficiency) at 10, 20, and 30 min, respectively, was 97.7, 97.4, and 96.6% for eugenol; 97.7, 96.6, and 95.9% for α-terpineol; 99.8, 99.5, and 98.8% for terpin-4-ol; and 97.3, 98.3, and 96.6% for methyl eugenol. With approximately a 10-min interval between application and feeding, the 10, 20, and 30 min estimates equate to the beginning, midpoint, and end of the 20-min test period. These data suggest volatile loss during the 20-min feeding was minimal. Untreated alfalfa pellets were also extracted and analyzed to determine background concentrations. None of the four compounds were present above GC/MS detection limits in untreated alfalfa pellets.

For each experiment, daily intake during the 20-min interval was analyzed as repeated measures using the MIXED procedure (SAS/STAT®, 2002–2003). The model included group as a fixed block effect, treatment, day, and day × treatment interaction. A compound symmetry, CS, covariance structure was used for repeated measurements on individual animals across days. Linear and quadratic models were fit to intake across concentrations averaged over days and across days averaged over concentrations when no day × treatment interaction was detected.

3. Results and discussion

No day × treatment interactions were detected in any experiment ($P > 0.05$). A day effect on intake ($P < 0.001$

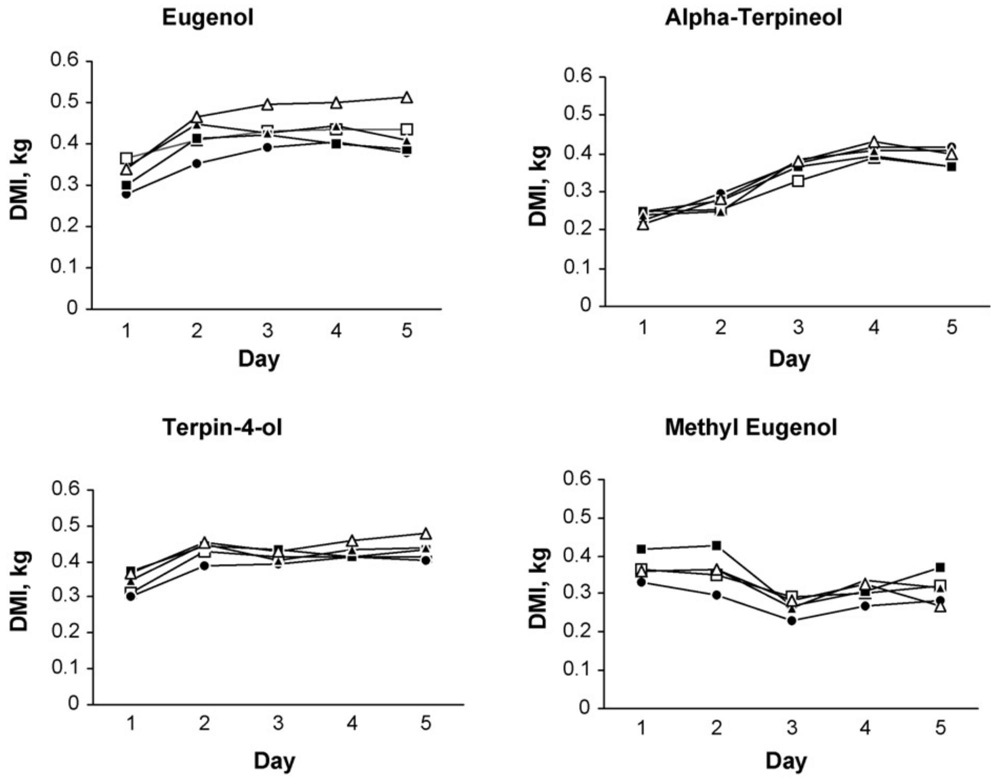

Fig. 1. Mean DM intake (DMI) of treated alfalfa pellets by lambs during a 20-min interval for five consecutive days; $N = 9$ lambs/d for each concentration; S.E.M. = 0.031, 0.030, 0.031, and 0.033 for eugenol, α-terpineol, terpin-4-ol, and methyl eugenol, respectively. Treatments (multiples of the concentration of specific compounds in tarbush) are designated (□), (●), (■), (▲), and (△) for 0×, 0.5×, 1×, 2×, and 10×, respectively. Linear and quadratic day effects ($P < 0.001$) pooled across treatments were observed for all four compounds.

for both linear and quadratic contrasts) was detected for all four chemicals, probably due to low intake at all concentrations (including controls; Fig. 1) on day 1 in all experiments except experiment 4 (methyl eugenol), which had greater intake initially. A general trend for lower intake during the first day or two (regardless of treatment concentration) was not surprising, given that similar linear and (or) quadratic effects were observed in 12 of 19 previous studies (Estell et al., 1998b, 2000, 2002, 2005). In contrast, the response in experiment 4 (higher intake at the beginning of the experiment) has not been observed in any of our previous studies. No evidence of post-ingestive aversion to volatile compounds was observed, given the lack of day × treatment interactions in any of the experiments (Fig. 1).

No treatment effect was detected ($P > 0.05$) for any of the compounds examined (Table 1). Although individual volatile compounds have been related to shrub herbivory by mammals in a few instances (e.g., sagebrush intake by mule deer [Personius et al., 1987], juniper intake by goats [Riddle et al., 1996], tarbush intake by sheep [Estell et al., 1998b, 2002], western red cedar intake by deer [Vourc'h

Table 1
Mean consumption (±S.E.M.) of alfalfa pellets treated with volatile compounds by lambs during a 20-min interval (kg/lamb/d, DM basis)

Concentration[a]	Eugenol[b]	α-Terpineol[b]	Terpin-4-ol[b]	Methyl eugenol[b]
0×	0.41 (0.031)	0.32 (0.030)	0.40 (0.031)	0.33 (0.033)
0.5×	0.36 (0.031)	0.35 (0.030)	0.38 (0.031)	0.28 (0.033)
1×	0.38 (0.031)	0.33 (0.030)	0.42 (0.031)	0.36 (0.033)
2×	0.42 (0.031)	0.34 (0.030)	0.42 (0.031)	0.33 (0.033)
10×	0.46 (0.031)	0.34 (0.030)	0.44 (0.031)	0.32 (0.033)

[a] Concentrations of volatile chemicals applied to alfalfa pellets were multiples (0, 0.5, 1, 2, or 10) of the concentration of that compound in tarbush (×); $N = 9$ lambs/concentration.
[b] A linear and quadratic day effect was observed in all four experiments ($P < 0.05$).

et al., 2002]), none of the compounds tested in the present study affected intake of alfalfa pellets. However, very little information is available regarding effects of these compounds on feeding by mammals. Riddle et al. (1996) reported α-terpineol was one of several monoterpenes that was negatively correlated to juniper intake by goats and Elliott and Loudon (1987) observed red deer rejected a pelleted diet when exposed to the odor of a mixture of five monoterpenes containing α-terpineol. However, neither of these studies examined α-terpineol independently of other chemicals, and the concentration to which animals were exposed differed. Reichardt et al. (1985) observed no effect of terpin-4-ol on feeding preference of hares. Cardoza et al. (2005) examined effects of eugenol on rumen fermentation, but we are aware of no studies that have examined effects of either eugenol or methyl eugenol on intake by mammals.

Only 4 of the 23 volatile compounds we have examined to date had a negative effect on intake by sheep. The general lack of response observed in these studies may be because cumulative and (or) synergistic effects of volatile compounds are more important than individual compounds. Total terpene concentration has been related to intake of ruminants previously (Riddle et al., 1996), and the total essential oil yield from tarbush (about 1% of DM; Tellez et al., 1997) is much greater than the 10× concentration of any chemical used in our studies. Some volatile compounds may simply serve as detection cues for other compounds (Lawler et al., 1999). Also, many studies showing terpenes to be related to dietary preferences examined animals on a low plane of nutrition. Secondary compounds are usually less aversive when fed with a high quality diet (Villalba and Provenza, 2005), and lambs in this study were fed a high quality diet at 3.7% of body weight. Finally, other classes of compounds may be partly responsible for the low palatability of tarbush.

4. Conclusions

None of the chemicals tested in this study were related to intake of alfalfa pellets by lambs under the conditions of this study. Other volatiles or compounds from other chemical classes may be responsible for the low preference of livestock for tarbush, and cumulative or synergistic effects of multiple compounds may also partly explain the lack of response to individual compounds. Information about the interactions of herbivores and specific plant chemicals will be crucial for development of mechanisms to alter foraging behavior and diet selection of livestock.

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