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EXTRACTS OF *Flourensia cernua* REDUCE CONSUMPTION OF ALFALFA PELLETS BY SHEEP[1]

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Abstract—Effects of three extracts (hexanes, ether, and ethanol) from tarbush (*Flourensia cernua*) on intake of alfalfa pellets by lambs were examined. Forty-five ewe lambs were fed one of five treatments for five days (randomized complete block, three lambs per block on each treatment). Treatments were alfalfa pellets (CON) or alfalfa pellets plus ethanol carrier (CAR), hexanes extract (HEX), ether extract (ETH), or ethanol extract (ETOH). Extracts were applied to alfalfa pellets at the same concentration as in an equivalent amount of tarbush (as fed basis) in experiment 1 and at 10-fold dilutions of that concentration in experiment 2. Treatments were isolated from tarbush leaves by using a sequential extraction with hexanes, diethyl ether, and 100% ethanol. Lambs received 640 g of alfalfa pellets (dry matter basis) each morning and intake was monitored during a 20-min interval. Lambs were maintained and fed alfalfa pellets (4.7% of body weight) as one group except during this interval. In experiment 1, mean intake by lambs during the 20-min interval was 361, 393, 204, 212, and 228 g for CON, CAR, HEX, ETH, and ETOH, respectively (SEM = 28.9). All three extracts decreased intake ($P < 0.001$) compared to CON or CAR. Intake did not differ among the three extracts (HEX, ETH, and ETOH) or between the two controls (CON and CAR). Mean intake did not differ among treatments in experiment 2 (468, 455, 389, 381, and 431 g for CON, CAR, HEX, ETH, and ETOH, respectively; SEM = 30.5; $P = 0.187$). Several compounds are probably

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responsible for the low palatability and differential use of tarbush typically exhibited by livestock.

Key Words—*Flourensia cernua*, feeding deterrent, intake, plant–animal interactions, plant extracts, sheep.

INTRODUCTION

Tarbush (*Flourensia cernua* DC) is a dominant shrub in the northern Chihuahuan Desert. It is high in crude protein and generally low in antiquality factors (except phenolics) (Estell et al., 1996). Livestock consume tarbush in limited amounts under free-ranging conditions (Nelson et al., 1970; Anderson and Holechek, 1983), depending on season and availability of other forages, but the shrub is not a preferred species. Tarbush may be toxic for some species of herbivores during flowering (Mathews, 1944; Dollahite and Allen, 1975). However, no adverse effects were observed in sheep consuming 30 or 15% tarbush leaves in mixed diets for 30 or 90 days, respectively (Fredrickson et al., 1994; King et al., 1996).

We have been using tarbush as a shrub model to examine chemical interactions between desert shrubs and mammalian herbivory. Ruminants exhibited differential use of individual plants when forced to browse tarbush (Estell et al., 1994b), and removal of surface compounds with organic solvents increased tarbush consumption by sheep (Estell et al., 1994a). Terpenes on the leaf surface of tarbush were related to its use by livestock (Estell et al., 1998a).

The objective of these experiments was to determine effects of extracts of tarbush on intake by lambs when applied to alfalfa pellets. Our hypothesis was that pellet consumption by lambs would decrease when extracts were applied.

METHODS AND MATERIALS

Experimental Protocol and Animal Management. Two experiments to examine intake of lambs consuming alfalfa pellets treated with extracts from tarbush were conducted in accordance with guidelines approved by the New Mexico State University Institutional Animal Care and Use Committee. Both experiments were part of larger studies. Experiment 1 was conducted in conjunction with a series of studies to examine the effect of specific terpenes (α-humulene, 1,8-cineole, p-cymene, 3-carene, and sabinene) on intake (Estell et al., 2000), and experiment 2 was part of a similar study that examined another series of terpenes (β-pinene, camphene, caryophyllene oxide, and myrcene; author's unpublished data). Both experiments were conducted as described by Estell et al. (2000). Ninety Polypay ewe lambs [45 lambs per experiment, approximately 5 months of age, mean initial body weight (BW) of 43.0 $\pm$ 0.41 kg and 36.8 $\pm$ 0.42 kg for experiments 1 and 2, respectively] without previous experience browsing tarbush were adapted to

alfalfa pellets [4.7% of BW, dry matter (DM) basis] for two weeks and a dry-lot pen for one week. A five-day adaptation period was conducted to familiarize lambs with handling procedures and the 20-min pen feeding (week 1), followed by a five-day period to establish baseline intake of untreated alfalfa pellets during the 20-min interval (week 2). Extracts were tested in week 8 (experiment 1) and week 7 (experiment 2). All five-day periods were separated by two-day intervals during which lambs were fed and managed as during the adaptation period.

Lambs were individually fed treated pellets each morning during a 20-min interval in an enclosed metabolism unit (1.22- × 2.44-m pens). Groups were fed in succession at 08:00, 08:30, and 09:00 hr. During the 20-min feeding, 640 g of pellets (DM basis) were offered daily to lambs, and feed refusals were measured. Except for the morning feeding period, lambs were maintained as one group in an outdoor pen with free access to water and trace-mineralized salt. Alfalfa pellets (≥15% CP, 0.95 cm diam., from sun-cured alfalfa hay) were sampled randomly, composited, ground to pass a 2-mm screen in a Wiley mill, and analyzed for dry matter (93.8% and 94.9% in experiments 1 and 2, respectively) (AOAC, 1990). Lambs were weighed on day 5 each week immediately preceding the 08:00 hr feeding.

Lambs were also group-fed untreated alfalfa pellets twice daily at 13:00 hr (950 g DM/lamb) and 10:00 hr (remainder required to provide a mean intake of 4.7% of BW). An amount of untreated feed equal to the total unconsumed feed during 20-min tests was also fed at 10:00 hr. During the two-day intervals between intake measurements, lambs were fed an additional 640 g DM/lamb at 10:00 hr.

Treatments. Treatments applied to alfalfa pellets consisted of extracts from an equivalent amount of tarbush (as fed basis, 680 g) in experiment 1. These concentrations should most accurately reflect those to which livestock would be exposed when browsing tarbush. For experiment 2, 10-fold dilutions of the extracts served as treatments. Two control groups (one receiving untreated pellets and one receiving pellets sprayed with ethanol carrier) were included in each experiment. Treatment extracts were thawed at room temperature for 24 hr, dissolved in the appropriate amount of ethanol, and stored in amber glass bottles. Extracts were not completely soluble in the amount of ethanol used, and some settling occurred. Consequently, suspensions were swirled immediately before decanting and application.

Solutions were applied at 1 ml/20 g of alfalfa pellets with graduated cylinders (experiment 1) or graduated high-density polyethylene spray bottles (experiment 2). Treatments were sprayed in a stream pattern in experiment 2 to minimize volatilization. Pellets were placed in one end of feed pans, and pans were tilted and gently shaken while treatments were applied in an effort to apply treatments completely and evenly. Approximately 10 min lapsed between application and feeding, and order of application was rotated systematically (within

and across days) to minimize bias due to time between spraying and feeding. Treatments were applied in an adjacent separately ventilated room, and an exhaust fan in the metabolism unit was used to remove aromas and minimize potential drift among pens. One stainless steel pan was used for each lamb in each experiment to minimize cross-contamination, and pans were rinsed with ethanol after each feeding.

Bulk Extraction. Tarbush exhibiting lush green growth (prebloom stage) without visible evidence of insect damage was harvested from one location on the Jornada Experimental Range near Las Cruces, New Mexico. Leaves were removed by hand using gloves and placed in plastic bags on ice. Approximately 36 kg of fresh material representing several hundred plants were collected on September 10, 1997, between 07:30 and 12:00 hr. Leaf material was frozen within 1–2 hr of collection, shipped on Dry Ice to the USDA-ARS Natural Products Utilization Research Unit (Oxford, Mississippi), and stored in a −20°C cold room. Frozen leaves were extracted sequentially with hexanes, diethyl ether, and 100% ethanol. Extractions were conducted in 3-kg batches at room temperature on a circular shaker at 150 rpm for 22 hr in a covered 10-liter round-bottom flask with 7 liters of each solvent. Extracts from each solvent were filtered (Whatmann No. 1 filter paper), solvents were removed with reduced pressure in a 10-liter rotary evaporator, and 12 extracts from each solvent were combined in amber glass bottles and frozen. Oils were stored in a −20°C cold room until shipped on Dry Ice to Las Cruces, New Mexico.

Statistical Analysis. Lambs were randomly assigned to pen, group, and treatment at the beginning of each experiment in a randomized complete block design. Randomization was restricted to three lambs per treatment in each group (block). Repeated-measures analysis of variance was conducted using GLM procedures of SAS Institute (1989) for each experiment to evaluate the consistency of intake among days within experiment. The model contained group (blocking factor), day, treatment, day × treatment interaction, and experimental error (animal nested within treatment). Orthogonal polynomial contrasts were tested among days in this analysis. Analysis of variance for each experiment was also conducted by using GLM procedures of SAS Institute (1989) with intake (five-day means) during the 20-min interval as the dependent variable and treatment and group as the independent variables in the model. Although pen and animal are confounded and pen was not in the model, previous experiments that used these facilities did not reveal pen effects (Estell et al., 1998b). Means from analysis of variance were separated ($P < 0.05$) by LSD (SAS Institute, 1989) in the case of a significant F value ($P < 0.05$). Intake of control lambs ($N = 9$) was subjected to analysis of variance with time as the treatment factor to evaluate the consistency of intake of controls over time. Means were separated ($P < 0.05$) by LSD (SAS Institute, 1989) in the case of a significant F value ($P < 0.05$).

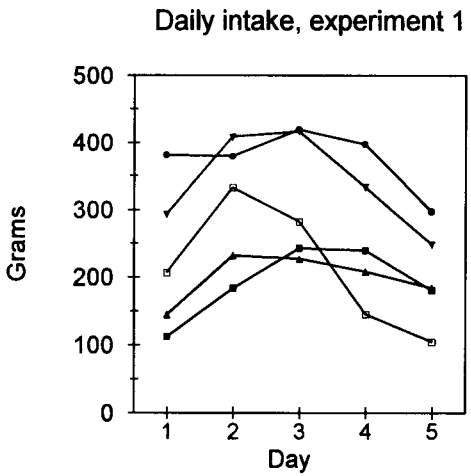

FIG. 1. Daily intake (g/day during 20 min feeding) by lambs of alfalfa pellets treated with *Flourensia cernua* extracts at the same concentration as in intact plants. Treatments were control (▼) and control with carrier (●), hexanes extract (■), ether extract (▲), or ethanol extract (□); $N = 9$ lambs/treatment; SEM = 28.9; linear treatment ($P < 0.001$) and quadratic mean ($P < 0.001$) effects were detected.

RESULTS AND DISCUSSION

When intake was examined by day with a repeated-measures analysis of variance, a linear treatment effect ($P < 0.001$) and a quadratic mean effect ($P < 0.001$) were detected in experiment 1 (Figure 1), and a quadratic ($P = 0.001$) mean effect was detected in experiment 2 (Figure 2). Quadratic mean effects in both experiments reflect the increased intake early in the week and the decrease in the latter part of the week. This shift was evident across treatments and therefore unrelated to treatments. The linear treatment effect in experiment 1 indicates changes in intake over time were not consistent among treatments (day × treatment interaction), and likely reflects the dramatic decrease in the ETOH treatment between days 2 and 5 (Figure 1).

Across days, intakes for CON (361 g) and CAR (393 g) differed ($P < 0.001$) from HEX (204 g), ETH (212 g), and ETOH (228 g) in experiment 1 (Figure 3). Although the pattern was similar in experiment 2 (Figure 4), means were not statistically different (468, 455, 389, 381, and 431 g for CON, CAR, HEX, ETH, and ETOH, respectively). All three treatments clearly reduced intake in experiment 1, supporting our hypothesis; however, the three fractions representing different crude mixtures of volatile and nonvolatile compounds extracted by increasingly polar

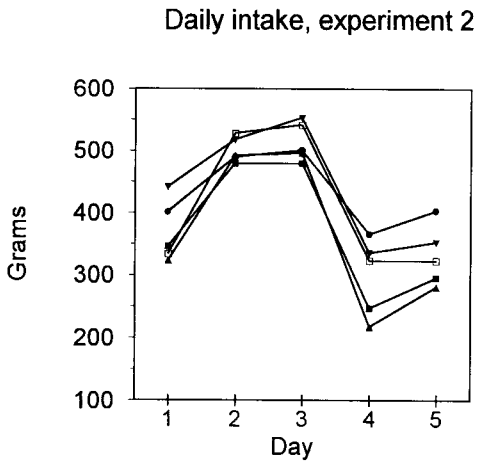

FIG. 2. Daily intake (g/day during 20 min feeding) by lambs of alfalfa pellets treated with *Flourensia cernua* extracts at a 10-fold dilution of the concentration in intact plants. Treatments were control (▼) and control with carrier (●), hexanes extract (■), ether extract (▲), or ethanol extract (□); $N = 9$ lambs/treatment; SEM = 30.5; linear ($P = 0.010$) and quadratic mean ($P < 0.001$) effects were detected.

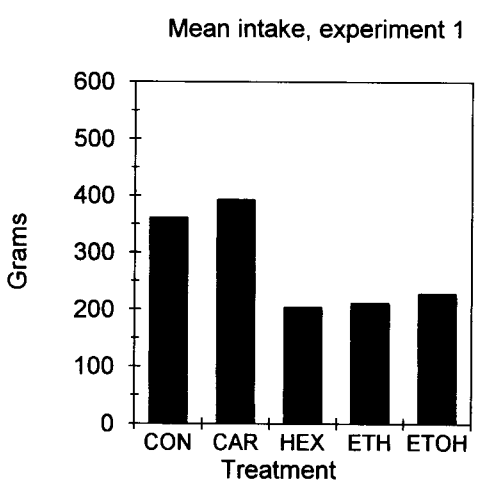

FIG. 3. Mean intake (g/day during 20 min feeding) by lambs of alfalfa pellets treated with *Flourensia cernua* extracts at the same concentration as in intact plants. Treatments were control (CON) and control with carrier (CAR), hexanes extract (HEX), ether extract (ETH), or ethanol extract (ETOH); $N = 9$ lambs/treatment; SEM = 28.9; CON and CAR differed from HEX, ETH, and ETOH ($P < 0.001$).

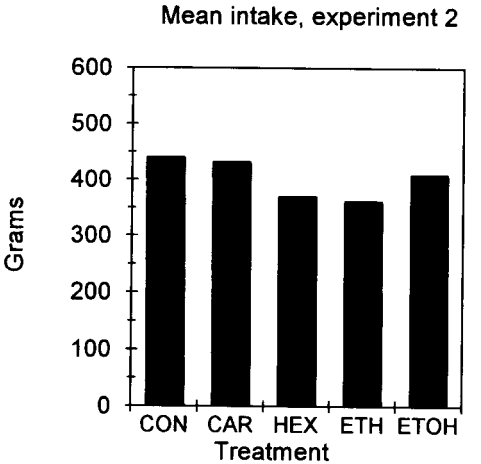

FIG. 4. Mean intake (g/day during 20 min feeding) by lambs of alfalfa pellets treated with *Flourensia cernua* extracts at a 10-fold dilution of the concentration in intact plants. Treatments were control (CON) and control with carrier (CAR), hexanes extract (HEX), ether extract (ETH), or ethanol extract (ETOH); $N = 9$ lambs/treatment; SEM = 30.5; treatment means did not differ ($P > 0.05$).

solvents were equally effective in deterring intake at concentrations equivalent to those encountered in intact plants.

Treatments CON and CAR were not different in experiments 1 or 2, indicating no effect of the ethanol carrier on intake of alfalfa pellets. No effect of group was detected in either experiment. Intake for the CON lambs during the 20-min interval was 361 g (experiment 1) and 468 g (experiment 2). Intake for the same nine lambs during the adaptation period (week 2) was 403 g and 536 g for experiments 1 and 2, respectively. Intake of control lambs during adaptation and week of treatment did not differ in either experiment ($P > 0.05$; SEM = 38.7 and 32.8, respectively). Because intake was adjusted weekly and because prior treatments did not greatly affect intake, we did not expect CON intake to differ over time.

Previously at this location, when a mixture of cattle, sheep, and goats were forced to use tarbush, they preferentially browsed certain plants to the exclusion of others, and this differential use was negatively related to epicuticular wax concentration (Estell et al., 1994b). Lambs consumed more tarbush when surface compounds were removed with organic solvents (Estell et al., 1994a). Concentrations of specific mono- and sesquiterpenes on tarbush leaves were related to this differential use (e.g., α-pinene and flourensadiol) (Estell et al., 1998a). To date, 15 volatile compounds (camphor, limonene, *cis*-jasmone, β-caryophyllene, borneol, α-pinene, sabinene, 3-carene, *p*-cymene, α-humulene, 1,8-cineole, camphene, myrcene, caryophyllene oxide, and β-pinene) have been tested individually

for effects on intake (Estell et al., 1998b, 2000, unpublished data). Results from these studies have generally shown minor or no effects on intake by lambs when individual compounds were applied to alfalfa pellets.

Because all treatments significantly reduced intake in experiment 1, a 10-fold dilution of the extracts was applied in experiment 2 in an attempt to identify the most potent fraction. None of the diluted extracts significantly influenced intake; however, the amount of residue recovered (415, 984, and 2215 g for hexanes, ether, and ethanol fractions, respectively) during extraction suggests the hexanes fraction was most potent, based on the fact that it was recovered in the lowest amount and, therefore, contributed the least mass to its respective treatment solution.

Volatile profiles for each extract were characterized by using gas chromatography–mass spectrometry (Tellez et al., 2001). Volatile profiles in the extracts were related more to molecule size than to any obvious polarity differences. The hexanes fraction contained most of the monoterpenes (and few sesquiterpenes), the ether fraction contained primarily sesquiterpenes (with some monoterpenes), and the ethanol fraction was almost exclusively sesquiterpenes. The volatile compounds contributing greater than 10% of the total peak area in the hexane fraction were limonene, myrcene, 3-carene, and β-eudesmol. For both the ether and ethanol fractions, only germacrene D and β-caryophyllene were present in excess of 10% of the total peak area (Tellez et al., 2001). Limonene, myrcene, 3-carene, and β-caryophyllene did not affect intake when examined individually (Estell et al., 1998b, 2000, unpublished data), although, to our knowledge, effects of germacrene D and β-eudesmol on mammalian herbivory have not been examined . These crude fractions likely also contain numerous nonvolatile compounds, particularly in the more polar ether and ethanol fractions (e.g., alkaloids, flavonoids, etc.). Flavonoids (Rao et al., 1970), 4-hydroxyacetophenone derivatives (Bohlmann and Grenz, 1977), and benzofurans and benzopyrans (Aregullin-Gallardo, 1985) have been characterized in tarbush. Certainly, these or other nonvolatile compounds in the extracts may be responsible for the reduced intake of alfalfa pellets by lambs in this study.

The decreased intake observed in experiment 1 may be due to reduced palatability, given that many compounds in the crude terpenoid fraction are bitter. Bitterness is thought to be a primary aversive stimulus influencing forage preferences (Krueger et al., 1974), although compounds considered to be bitter are not necessarily deterrent to herbivores (Nolte et al., 1994). Hanks et al. (1975) reported that methanolic extracts containing phenolics from a number of subspecies of rubber rabbitbrush, varying in palatability and use by mule deer, resulted in different patterns when subjected to paper chromatography. Dohi et al. (1996) found methanolic extracts of perennial ryegrass sprayed on low-quality hay stimulated intake by goats, whereas pentane extracts either had no effect or reduced intake. Pass et al. (1998) examined the deterrent properties of eucalyptus for

common ringtail possums, using plants that were herbivore deterrent and herbivore susceptible. Several fractions from low-use plants differed from controls, including a methanolic fraction, a chloroform subfraction, and the steam distillate (Pass et al., 1998).

Intake reductions could be attributed to reduced digestibility and or passage rate due to microbial toxicities. Terpenes have been reported to decrease *in vitro* digestibility in ruminants, suggesting microbial toxicities (Oh et al., 1967; Schwartz et al., 1980), particularly if rumen microflora are not adapted to the compounds (Oh et al., 1967). Sinclair et al. (1988) indicated both dry matter and protein digestibility in snowshoe hares were reduced when ether or methanol extracts of white spruce or bog birch were applied to rabbit chow at levels found in those species normally. However, it is doubtful that reduced digestibility was responsible for decreased intake in this study, given the short duration of the study and the fact that lambs maintained an average total intake of 4.7% of BW.

There was little indication that intake reduction was due to negative feedback in this study, even though aversions can be formed within a five-day period (Provenza et al., 1990). The only evidence of aversion to extracts occurred for the ETOH treatment (experiment 1; Figure 1), in which intake decreased substantially during the latter part of the week (linear treatment effect, suggesting a day $\times$ treatment interaction, $P < 0.001$).

In conclusion, compounds in these extracts are probably partly responsible for the low palatability and differential use of tarbush by ruminants. Knowledge of specific chemical interactions with feed intake may ultimately lead to mechanisms to alter feeding behavior and diet selection. Potential benefits of increased shrub consumption would depend on the balance of nutritional benefits and metabolic impacts of phytotoxins.

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