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INFLUENCE OF FLAVOR ON GOAT FEEDING PREFERENCES

G. DE ROSA,[1,*] L. MOIO,[2] F. NAPOLITANO,[3] F. GRASSO,[1] L. GUBITOSI,[1] and A. BORDI[1]

[1]*Dipartimento di Scienze zootecniche e Ispezione degli alimenti*

[2]*Dipartimento di Scienza degli Alimenti*
Università degli Studi di Napoli "Federico II"
Via Università 133, 80055 Portici (NA), Italy

[3]*Dipartimento di Scienze delle Produzioni Animali*
Università degli Studi della Basilicata
Via N. Sauro 85, 85100 Potenza, Italy

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Abstract—Goat feeding preferences for straw pellets flavored with ryegrass (*Lolium perenne*, cv. Belida) or white clover (*Trifolium repens*, cv. Huia) aromatic extracts, obtained by means of a cold aromatic extraction method, were assessed with cafeteria trials. Prior to the trials, odor differences between the two plant species, the two aromatic extracts, and the straw pellets sprayed with the two aromatic extracts were verified using sensory analysis performed by 30 human panelists. Since odor differences observed among fresh samples were still detectable in aromatic extracts and moistened pellets, the extraction method was considered effective in reproducing plant odors. Straw pellets sprayed with either distilled water (W) or ryegrass (R) or clover (C) aromatic extracts were used to assess flavor preferences of 12 female Rossa Mediterranea goats. Sprayed pellet preference was evaluated in two sessions conducted in two consecutive weeks. Each session consisted of three two-choice presentations performed on three consecutive days. In both sessions, food intake, proportion of food intake, time spent feeding, and proportion of time spent feeding were significantly affected by pellet type ($0.05 > P > 0.001$). In particular, straw pellets sprayed with ryegrass extract were highly selected compared to those sprayed with clover ($0.01 > P > 0.001$) or water ($0.01 > P > 0.001$). In addition, in the second session, the clover extract was preferred to distilled water ($0.05 > P > 0.01$). The results of this study gave two main indications: first, goat selectivity for ryegrass against clover was consistent even when straw pellets sprayed with odors of these plants were offered, and secondly, the addition of aromatic extracts to straw pellets increased the preference for pellets.

*To whom correspondence should be addressed.

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Key Words—Ryegrass, clover, goats, food preferences, flavor, aromatic extracts, cafeteria trials.

INTRODUCTION

In temperate grassland, goats actively select grasses over legumes. This results in increased clover content in goat-grazed pastures as compared with those grazed by sheep (Radcliffe and Francis, 1988; Bown et al., 1989; del Pozo and Wright, 1995). Lambs grazing after goats consume diets with higher clover content and show higher growth rates than those grazing after sheep (del Pozo and Wright, 1995). Goat selectivity for grasses as opposed to legumes is also consistent in short-term trials (De Rosa et al., 1997). Although levels of non-structural carbohydrates are equivalent between legumes and grasses, legumes contain a much lower proportion of water-soluble carbohydrate (Smith, 1973; Osbourn, 1980). The different dietary preferences observed in sheep and goats may be due to the fact that goats ingest plants with high non-structural carbohydrate content (Fedele et al., 1993), whereas plants with higher levels of secondary metabolites such as tannins, alkaloids, terpenes, and cyanogenic glycosides (e.g., legumes) are disliked (Narjisse, 1991; Morand-Fehr et al., 1993; Narjisse et al., 1996).

The forage preference is considered to be a function of taste, odor, texture (forage flavor), and postingestive effects on the animal (Provenza, 1995, 1996). Animals acquire preferences for nutritious foods and dislike foods with low nutrient content or high toxins levels (Provenza, 1995, 1996).

Goats learn to avoid condensed tannins from the current season's twigs of blackbrush (*Coleogyne ramosissima*) by associating the flavor of foods containing condensed tannins with aversive postingestive effects (Provenza et al., 1990). Sheep and goats appear to discriminate against monoterpenoid odor and taste, respectively, but this possibility has not been fully ascertained (Narjisse et al., 1996). Sheep regulate intake according to the flavor concentration of the feed, when flavor and toxicity are strongly correlated (Launchbaugh et al., 1993). In contrast, when they are not associated, animal intake relied on the maximum dose of toxin they had ingested (Launchbaugh et al., 1993). Sheep also more readily eat an unfamiliar feed associated with a familiar flavor or odor, whereas they avoid it when presented with a novel odor (Van Tien et al., 1998). Moreover, it has been shown that in sheep, hay palatability may be modified using different chemical compounds. The intake of wheaten hay was increased by the addition of butyric acid and monosodium glutamate, whereas it was reduced by MgO (Gherardi and Black, 1991; Gherardi et al., 1991). These results indicate that the flavor of the forage may play an important role in determining herbivore feeding preferences.

The present study aimed to evaluate the importance of flavor on feeding preferences of goats. We developed a cold aromatic extraction method for ryegrass

(*Lolium perenne*, cv. Belida) and white clover (*Trifolium repens*, cv. Huia) herbage that was effective in reproducing the corresponding plant's odor characteristics. Feeding preferences can be assessed by either long-term trials, comparing the proportion of a plant species in the diet as against its proportion in the sward, or cafeteria trials, directly testing preferences using two-choice presentations (Gordon, 1995). Goat feeding preferences for the aromatic extracts sprayed on straw pellets were assessed using cafeteria trials. The plants were chosen according to their different palatability as shown by Fedele et al. (1993) in field trials conducted in a Mediterranean grazing environment and by De Rosa et al. (1997) in cafeteria trials, who noted that ryegrass was highly selected, whereas clover was disliked by goats.

METHODS AND MATERIALS

Odor Differences between Ryegrass and White Clover. Prior to preparation of aromatic extracts from ryegrass and white clover, odor differences between the two plant species were verified using sensory analysis. Thirty human panelists, (students and staff members of the Department of Animal Production, University of Naples), were selected on the basis of their availability and motivation. Triangle tests were performed, offering each member two sets of three samples of herbage in the vegetative stage harvested just before use and chopped into 4- to 6-cm pieces. The weight of each sample was 30 g. Two samples were alike and one was different within each triangle test. Samples were presented in Teflon bags that lay on a layer of ice placed in Pyrex beakers to avoid olfactory bias and visual discrimination. Panel members were asked to identify the odd sample. Triangle tests were performed in a 25-m^2 room where the panel members were individually introduced to the person conducting the test. Three sessions of the triangle test were performed per panelist.

Preparation of Aqueous Aromatic Extracts. Samples from the two plant species were harvested and immediately frozen in liquid nitrogen in order to minimize degradation and/or transformation of odorous molecules. Frozen plants were powdered in liquid nitrogen and added to distilled water at 50, 100, or 200 g/liter. Powder and water were mixed for 48 hr at 4°C. Subsequently, the aqueous solution containing volatile components was centrifuged for 10 min at 7000 rpm and stored at −80°C until use. A sensory test was conducted to determine the concentration of the aromatic extract that most resembled the corresponding plant smell. Extracts were thawed overnight at 4°C and introduced in 50-ml flasks wrapped with aluminum foil. Human panelists were asked to associate, by sniffing, a series of different concentrations of aromatic extract (5, 10, and 20%) to the original plant species. For each plant species, panelists received three samples of extracts and a sample of corresponding herbage. Three sessions of this test (the similarity test) were performed. The panelists chose the 10% powder concentration for

both ryegrass and clover as most similar to the corresponding plant species (see Results). Therefore, this concentration was used for the successive experimental steps.

Subsequently, three triangle test sessions for aqueous aromatic extracts of ryegrass and clover were performed as described above to test whether odor differences between the two plant species were consistent in the corresponding aromatic aqueous solutions after the cold extraction.

Preparation of Samples Offered to Animals. Straw pellets are characterized by a low-intensity odor that is cosidered unlikely to interfere with the volatile components of an aqueous solution. Straw pellets are also known to be disliked by goats, so that any increase or decrease in consumption may be attributed to the effect of extract addition. Thus, the aromatic extracts to be offered to the goats were incorporated into straw pellets. Three sessions of triangular tests where the straw pellets were sprayed with ryegrass or clover aromatic extracts (50 ml of aqueous solution for 200 g of straw pellet) were performed.

Experimental Design. Twelve female Rossa Mediterranea goats were used in this experiment. The age and weight of the animals were 2–3 years and 35–45 kg, respectively. The goats were not pregnant or lactating, and all had previous experience of consuming both plant species.

The goats were equally divided into two groups and group-housed in two straw-bedded pens (4 $\times$ 2 m) with water available *ad libitum*. The animals were exposed to a three-week preliminary phase, during which they were offered straw pellets ad libitum in plastic baskets (50 $\times$ 34 $\times$ 15 cm) from 09:00 to 14:00 hr and, subsequently, mixed grass hay *ad libitum*.

In a subsequent two-week training phase, the animals were moved once a day into individual test pens (1.2 $\times$ 2 m) for 10 min where they received approximately 800 g of straw pellets in four plastic baskets placed in a wooden trough located 30 cm above floor level. An observer sat in front of each individual test pen to acclimate the animals to human presence. The goats in the adjacent test pens could not see each other. Thereafter, the animals were moved to the home pen where *ad libitum* mixed grass hay was offered. In the last two days of the training phase, straw pellets were sprayed with distilled water (50 ml/200 g).

Cafeteria Trials. During the preference tests, straw pellets sprayed (50 ml/200 g) with either distilled water (W) or ryegrass (R) or clover (C) aromatic extracts were used to assess goat flavor preference. The pellets were sprayed immediately prior to the start of the preference tests.

For each goat, sprayed pellet preference was evaluated in two sessions performed in two consecutive weeks. Each session consisted of three two-choice presentations conducted over three consecutive days. Two-choice presentations started at 09:00 hr and never continued past 12:00 hr. Five minutes before each presentation, the animals were moved to the individual test pens. The animals were offered two samples (sprayed pellets) simultaneously for 3 min in four adjacent

baskets (200 g/basket) filling all the through front. Two consecutive baskets contained sample 1 and two contained sample 2. On test days, each pair of samples (ryegrass–water, clover–water, and ryegrass–clover) was offered to four animals to avoid day effect. Three goats from the same group were simultaneously tested, always at the same time of day, with each animal getting a different pairing. Goats were used for only one two-choice presentation a day. Samples were assessed with the same frequency in each spatial (left–right) and temporal position (ryegrass–water, clover–water, ryegrass–clover), thereby minimizing the effects of position habits and order of presentation. Samples were weighed before and after each presentation in order to determine the total intake using an electronic digital scale. Six control baskets (two for each sample) were used to estimate the evaporative weight losses and the total intake was corrected accordingly. During the two-choice presentation an observer recorded feeding time (prehension time + mastication time) for each sample. For each session, the preference of the animals for each sample was determined by using the averages of time spent feeding, food intake, proportion of time spent feeding (time spent feeding on one sample/total feeding time), and proportion of food intake (intake from one sample/total intake) recorded in all the two-choice presentations where the sample was present (Morand-Fehr et al., 1987). After the two-choice presentations, the animal were moved to the home pen and fed with mixed grass hay *ad libitum*.

Statistical Analyses. Data were analyzed with a statistical package for social sciences (SPSS-PC+, version 3.1) (Norusis, 1989). Data obtained from human sensory evaluation were analyzed with the χ^2 one-sample test, where the expected frequencies of the odd samples (triangle tests) and of each concentration of aromatic extract (similarity tests) were 33.3%.

For the cafeteria data, the goat was used as the experimental unit. For each session of cafeteria trial, the variables collected (time spent feeding, food intake, proportion of time spent feeding, and proportion of food intake) were analyzed using the Friedman two-way analysis of variance by ranks test with pellet type (R, C, and W) as factor. When significant effects were recorded, the differences among the means were evaluated by the Wilcoxon matched-pairs signed-ranks test. The latter was also used to test session effect for each type of pellet.

RESULTS

Validation of Extraction Technique. Triangular tests performed on the two plant species, their aromatic extracts, and moistened pellets showed significant differences between clover and ryegrass odors ($P < 0.001$) (Table 1). Since odor differences observed between fresh samples were still detectable in aromatic extracts and moistened pellets, the extraction method can be considered effective in reproducing plant odors.

TABLE 1. DEGREE OF DISCRIMINATION OBSERVED DURING TRIANGLE TESTS OF RYEGRASS AND CLOVER OFFERED AS HERBAGE, AROMATIC EXTRACTS, AND SPRAYED STRAW PELLETS TO 30 PANELISTS

Sample	Comparison	Session	Proportion of correct responses	Significance[a] (P)
		I	0.83	<0.001
Herbage	ryegrass–clover	II	0.83	<0.001
		III	0.87	<0.001
		I	0.87	<0.001
Aromatic extract	ryegrass–clover	II	0.85	<0.001
		III	0.73	<0.001
		I	0.72	<0.001
Sprayed pellets	ryegrass–clover	II	0.75	<0.001
		III	0.80	<0.001

[a] χ^2 one-sample test; $df = 1$.

Similarity tests were conducted to evaluate the concentration of aqueous extracts that presented the odor closest to the corresponding plant (Table 2). Results indicated that a 10% powder concentration could be considered to be the most similar to the corresponding plant, since the choice of assessors for this concentration over the three sessions was 55.6% and 55.7% for ryegrass and clover, respectively ($\chi^2 = 7.02$, $df = 2$, $P < 0.05$ and $\chi^2 = 8.6$, $df = 2$, $P < 0.05$, respectively). Therefore, this concentration was used in cafeteria trials.

Cafeteria Trials. Food intake (Figure 1) was significantly affected by pellet type in both sessions ($\chi^2 = 6.5$, $df = 2$, $P < 0.05$ and $\chi^2 = 13.54$, $df = 2$, $P < 0.001$, respectively). In the first session, R consumption was higher than C and W

TABLE 2. SIMILARITY OBSERVED BY 30 PANELISTS BETWEEN HERBAGE AND CORRESPONDING AROMATIC EXTRACT

Herbage	Concentration of aromatic extract (%)	Proportion of responses per session[a]		
		I	II	III
	5	0.20	0.13	0.20
Ryegrass	10	0.53	0.54	0.60
	20	0.27	0.33	0.20
χ^2		5.6*	7.2*	9.6**
	5	0.27	0.33	0.30
Clover	10	0.60	0.54	0.57
	20	0.13	0.13	0.13
χ^2		10.4**	7.2*	8.6*

[a] χ^2 one-sample test; $df = 2$; *$P < 0.05$; **$P < 0.01$.

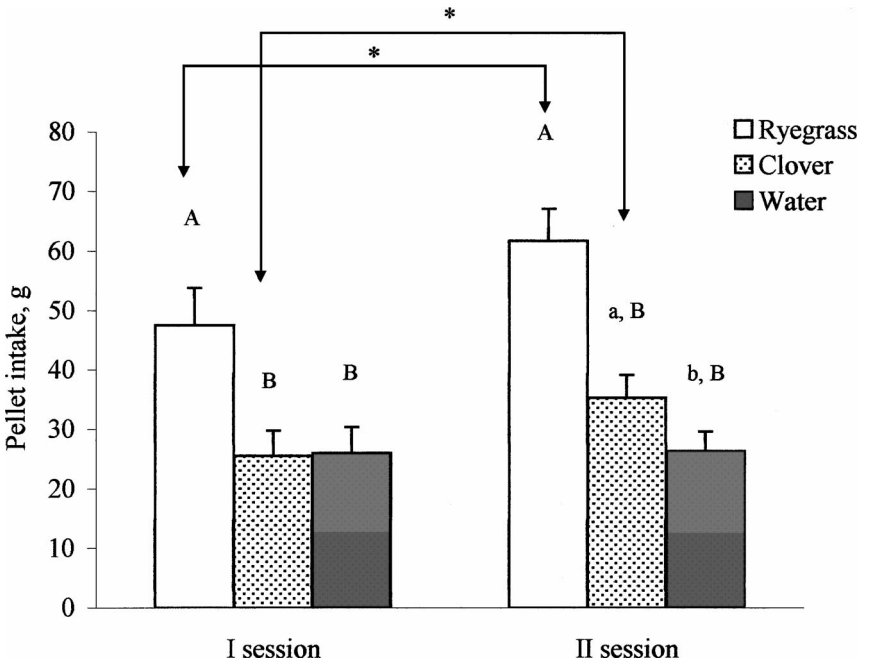

FIG. 1. Goat intake (mean + SE) of straw pellets sprayed with either distilled water or ryegrass or clover aromatic extracts in the two session cafeteria trial. A, B: means within sessions followed by a different letter are different at $P < 0.001$. a, b: means within sessions followed by a different letter are different at $P < 0.05$. $*$ means between sessions are different at $P < 0.05$.

($P < 0.001$). There was no difference between C and W. In the second session, goats showed a higher intake for R than for C and W ($P < 0.001$), as well as a higher ingestion of C than W ($P < 0.05$). A comparison between the two sessions showed higher intakes for R and C in the second trial ($P < 0.05$), but no differences between trials for W. Similar results were observed for the proportion of food intake except for the differences between trials (Figure 2).

For both sessions a significant effect of pellet type on time spent feeding ($\chi^2 = 6.5$, $df = 2$, $P < 0.05$ and $\chi^2 = 17.17$, $df = 2$, $P < 0.001$, respectively; Figure 3) was found. In fact, in the first session, the animals spent more time eating R than C and W ($P < 0.01$). There were no differences observed between C and W. In the second session, time spent feeding on R was higher than that spent on C and W ($P < 0.01$), whereas this variable was lower for W than for C ($P < 0.01$). A comparison between the two sessions showed higher levels of time spent feeding on W in the first session ($P < 0.05$). Accordingly, the proportion of time spent feeding showed a similar pattern (Figure 4).

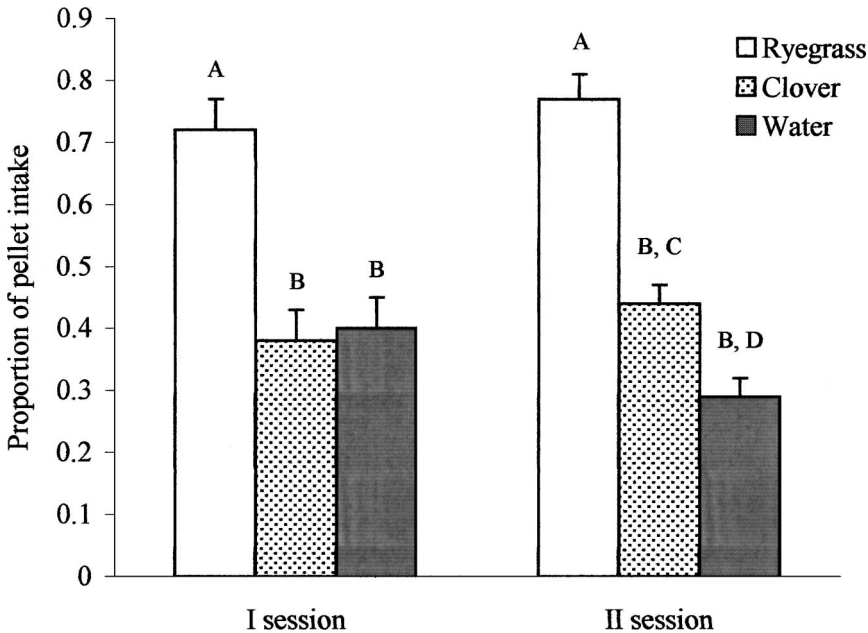

FIG. 2. Proportion of goat intake (mean + SE) of straw pellets sprayed with either distilled water or ryegrass or clover aromatic extracts in the two session cafeteria trial. A, B: means within sessions followed by a different letter are different at $P < 0.001$. C, D: means within sessions followed by a different letter are different at $P < 0.01$.

DISCUSSION

In this study, plant physical characteristics were removed by transferring the odorous molecules from ryegrass and clover to aqueous solutions. However, the aroma of the original sample extract may change during the extraction process and does not necessarily reproduce the characteristics of the original sample from which it was obtained (Moio et al., 1995). If such an odor distortion occurs during the preparation of extract, the aromatic aqueous phase offered to the animals may exhibit olfactory properties different from those of the original plant. Therefore, the first objective of this study was to develop an extraction procedure yielding extracts with odors close to those of the original plant. The results of sensory analysis indicate that the cold aromatic extraction technique from ryegrass and white clover may be effective in reproducing the corresponding plant odor characteristics. The importance of using a low temperature during the extraction process to attain a good representation of the extracts before sensory analysis has also been reported for wine (Moio et al., 1995), cheese, and tomato (Etievant et al., 1995).

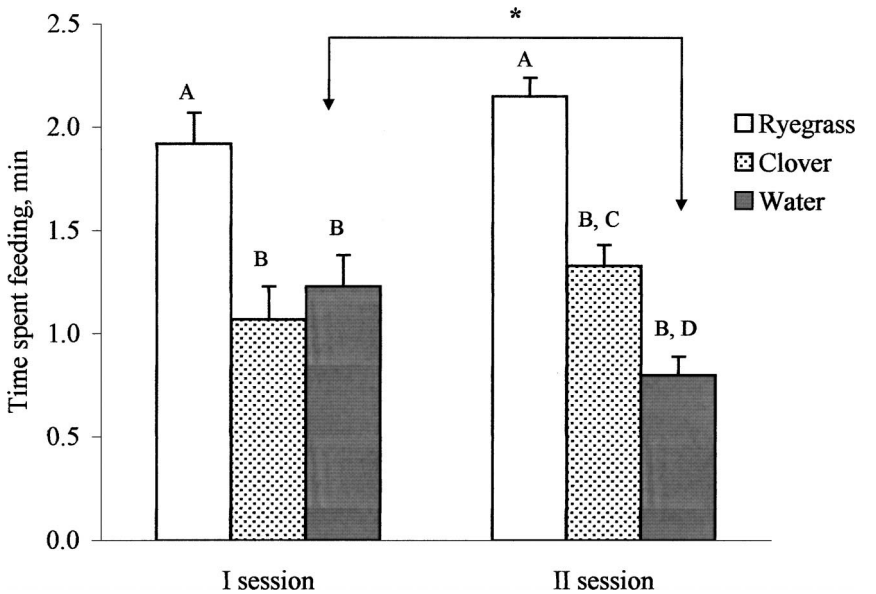

FIG. 3. Time spent feeding (mean + SE) by goats on pellets sprayed with either distilled water or ryegrass or clover aromatic extracts in the two session cafeteria trial. A, B and C, D: means within sessions followed by a different letter are different at $P < 0.01$. $*$ means between sessions are different at $P < 0.05$.

The results obtained in the cafeteria trials with the four dependent variables (time spent feeding, food intake, proportion of time spent feeding, and proportion of food intake) were generally similar. This study indicates that the addition of aromatic extract (ryegrass or clover) to straw pellets usually increased the preference for pellets. In particular, the intake of straw pellets sprayed with ryegrass extract was higher than those sprayed with water in both sessions, whereas for the clover extract this was observed only in the second session. These findings are in agreement with Provenza et al. (1996), who suggested that offering lambs the same food in different flavors influences food intake, as does offering different foods of similar or different nutritional value.

In field experiments, it has been found that ryegrass and white clover swards grazed by goats have more clover than those grazed by sheep (Penning et al., 1996; del Pozo et al., 1998). In a Mediterranean grazing environment, where the vegetation was exclusively herbaceous, Fedele et al. (1993) found that goats preferred grasses and had little interest in forbs and legumes. A preference for grasses over legumes was also confirmed in short-term trials (De Rosa et al., 1997). It has been suggested that goats prefer plants with high non-structural carbohydrate content (Fedele et al., 1993), whereas plants with higher levels of secondary metabolites,

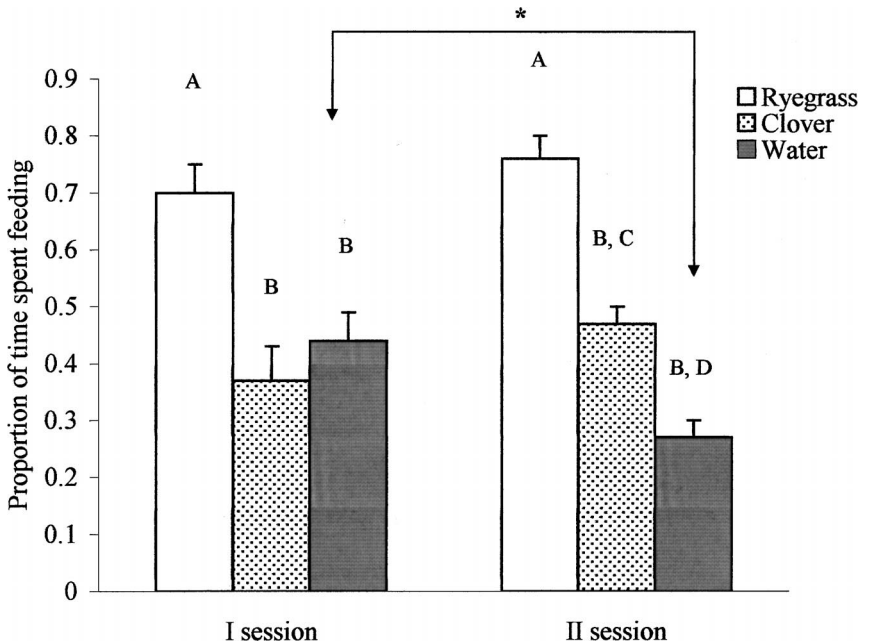

FIG. 4. Proportion of time spent feeding (mean + SE) by goats on straw pellets sprayed with either distilled water or ryegrass or clover aromatic extracts in the two session cafeteria trial. A, B and C, D: means within sessions followed by a different letter are different at $P < 0.01$. $*$ means between sessions are different at $P < 0.05$.

which are considered protective agents for plants against herbivores and pathogens (Provenza et al., 1988) such as tannins, terpenes, alkaloids, cyanogenic glycosides (e.g., legumes) are markedly disliked by goats (Narjisse, 1991; Morand–Fehr et al., 1993). In fact, removal of surface compounds with organic solvents increased tarbush (*Flourensia cernua*) intake by sheep (Estell et al., 1994), and terpenes on the leaf surface were related to diet preference (Estell et al., 1996). Accordingly, diet selection of goats was inversely correlated with tannins (Provenza and Malechek, 1984; Provenza et al., 1990). Goats were also able to taste monoterpenes (Narjisse et al., 1996) in pellets, and a positive (camphor) or negative (α-pinene, limonene) correlation between the content of these monoterpenes with juniper ingestion has been found (Riddle et al., 1996). Conversely, in lambs a detrimental effect of both camphor and α-pinene concentration on pellet intake has been reported (Estell et al., 1998). These findings are consistent with our results where the preference was higher for ryegrass extract than for clover, although the nutritional value of the two pellets was similar and the factor of plant physiological characteristics was removed. Therefore, only sensory cues (odor and taste) could be used by goats for

pellet discrimination, thus confirming that food flavor may play an important role in affecting goat preferences.

Food preferences depend on the functional interrelationship between flavor and postingestive effects of nutrients (Provenza et al., 1996). Animals discriminate among foods using taste, odor, and sight, which are a source of hedonic sensations (Provenza, 1996). They acquire preference for nutrients and for high levels of rapidly digestible nutrients and an aversion for toxins and foods deficient in specific nutrients (Provenza, 1995, 1996). It can be argued that in the present study goat selection was not based on postingestive effects, since short-term trials were performed. Thus, the data may reflect only the initial response of goats to the flavors of ryegrass and clover. The animals were exposed to these flavors after one week and the preference for ryegrass over clover was confirmed. The animals used in this study had experienced grazing in open rangeland, where they may have learned to associate a particular flavor with negative (or positive) postingestive effects in previous grazing activity. This hypothesis is supported by experimental evidence that young mammalian herbivores can still associate, even after as long as three years, negative (Burritt and Provenza, 1990; Distel and Provenza, 1991) or positive associations (Squibb et al., 1990; Distel and Provenza, 1991) with specific foods. It is likely that the goats generalized preferences and aversions to the flavored straw pellets on the basis of their past experiences with straw, clover, and ryegrass. Flavor generalizations have been demonstrated for food preferences (Ralphs et al., 1995) and food aversions (Launchbaugh and Provenza, 1993). This is especially likely to be the case given the limited amount of time the goats were tested. With more time and greater intake of the pellets, the postingestive effects of straw would likely have played a greater role in influencing food selection (Lawler et al., 1999; Villalba and Provenza, 2000). In addition, during the cafeteria trials, the goats ate limited amounts of the straw pellets, less than 50 g of each flavored pellet, and they were offered the foods briefly (3 min) on only six occasions. Food items that make up such a small part of the daily ration have relatively little postingestive influence on food preference (Villalba and Provenza, 2000).

Results from this study show that goat selectivity for ryegrass as opposed to clover is consistent, even when straw pellets sprayed with odors of these plants are offered. Further studies using gas chromatography–sniffing and gas chromatography–spectrometry analysis to determine the aromatic compounds responsible for grass–legume discrimination and preference are the next steps, because a better understanding of the mechanisms underlying food preferences may lead to strategies for altering diet selection.

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