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RUMINAL METABOLISM OF LEAFY SPURGE IN SHEEP AND GOATS: A POTENTIAL EXPLANATION FOR DIFFERENTIAL FORAGING ON SPURGE BY SHEEP, GOATS, AND CATTLE[1]

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Abstract—Leafy spurge (*Euphorbia esula*) is an introduced forb that is invading western rangelands. Goats (*Capra hircus*) readily graze the plant, but cattle (*Bos tarus*) generally and sheep (*Ovis aries*) locally appear to develop conditioned flavor aversions to leafy spurge. They either avoid the plant entirely or graze it reluctantly. We hypothesized that: (1) a diterpene diester that can occur in leafy spurge was an aversive agent, and (2) diet selection differences among ruminant species may be partly a function of differential ruminal metabolism of aversive phytochemicals, and further that cattle and sheep may be reluctant to graze leafy spurge because their ruminal microbes do not metabolize certain leafy spurge chemicals as do ruminal microbes in goats. Sheep did not develop an aversion to a novel food when its consumption was followed by an intravenous injection of ingenol 3,20-dibenzoate ($P = 0.34$). Sheep did develop an aversion to a novel food when its intake was followed by a dose of leafy spurge fermented with sheep ruminal digesta, but not when followed by a dose of leafy spurge fermented with goat ruminal digesta ($P = 0.03$). This suggests that goat ruminal microbes may modify leafy spurge such that it does not elicit an aversion in sheep.

Key Words—Conditioned flavor aversion, diet selection, ruminants, phytotoxins, ingenol, weeds, leafy spurge, *Euphorbia esula*, ruminal metabolism, sheep, *Ovis aries*, goat, *Capra hircus*, cattle, *Bos tarus*.

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INTRODUCTION

In contrast to goats, cattle and sheep can develop feeding aversions to the introduced rangeland and pasture weed leafy spurge (*Euphorbia esula*) (Kronberg et al., 1993a). This finding is consistent with field observations that cattle generally do not graze the plant (Lacey et al., 1985; Lym and Kirby, 1987) and mixed reports that sheep will or will not readily graze it at least in some areas of its infestation (Landgraf et al., 1984; Lacey et al., 1984; Bartz et al., 1985; Kronberg et al., 1992). The finding is also consistent with our field observations in southeastern Idaho that goats graze leafy spurge more willingly than do sheep (Walker and Kronberg, 1992). Our findings suggest that leafy spurge contains one or more chemicals that can elicit an aversive response in cattle and sheep (Kronberg et al., 1993a,b).

The identity of the aversive chemical(s) in leafy spurge is unknown. However, various diterpenoid ingenols have been found in the plant (Kupchan et al., 1976; Upadhyay et al., 1978; Seip and Hecker, 1982; Sorg and Hecker, 1982). Kupchan et al. (1976) found that the diterpene ingenol 3,20-dibenzoate from leafy spurge had inhibitory activity against lymphocytic leukemia in mice, and Sorg and Hecker (1982) found that it had high inflammatory activity on mouse ears. Upadhyay et al. (1978) found two ingenols in leafy spurge that had skin irritant and inflammatory properties, and Seip and Hecker (1982) found a fraction of leafy spurge that exhibited irritant and weak tumor-promoting properties. We suspect that the aversion-eliciting characteristic of leafy spurge may be due to its diterpenoid compounds for two reasons. First, chemicals with cytotoxic activity are typically aversive (Seynaeve et al., 1991; Mattes et al., 1992), and secondly, both lithium and phorbol 12-myristate 13-acetate (PMA) inhibit the gene expression for phosphoenolpyruvate carboxykinase, a key enzyme in gluconeogenesis (Bosch et al., 1992; Chu and Granner, 1986). Lithium is an aversive agent widely used in the study of taste aversion learning (Riley and Tuck, 1985), and PMA and ingenols are related diterpenes (Evans, 1986).

We suspect that ingenol is absorbed into the blood from the gastrointestinal tract. Once in the blood, it reaches the area postrema in the medulla oblongata of the brain, where a chain of events leads to the development of a conditioned flavor aversion (Borison, 1986; Kosten and Contreras, 1989) for leafy spurge.

Diet selection differences among ruminant species may be partly a function of differential ruminal metabolism of phytochemicals. Ruminal microbes can detoxify or produce toxins from phytochemicals (James et al., 1975; Allison et al., 1981, 1990; Carlson and Breeze, 1984; Craig et al., 1992). Rumen microbial populations in different ruminant species can have critically different capacities for degrading plant toxins (Wachenheim et al., 1992); therefore, we rationalized that cattle and sheep may be reluctant to graze leafy spurge because

their ruminal microbes do not metabolize aversive leafy spurge phytochemicals as do ruminal microbes in goats.

The objectives of our study were to learn if one of the ingenols isolated from leafy spurge could elicit an aversive response in sheep and to determine if processes in the rumen might account for interspecific differences in preference for leafy spurge among cattle, sheep, and goats. Specifically, we tested two hypotheses: (1) sheep will develop aversions to a novel food when intake of the food is followed by an intravenous dose of ingenol 3,20-dibenzoate, and (2) sheep will develop aversions to a novel food when intake of the food is followed by leafy spurge that is fermented with sheep ruminal microbes, but not when intake of the novel food is followed by a dose of spurge that is fermented with goat ruminal microbes.

METHODS AND MATERIALS

Experiment 1. This experiment studied the aversiveness of ingenol 3,20-dibenzoate to sheep. Ten weaned white-face lambs (mean wt, 31 $\pm$ 3 kg) were randomly divided into two treatment groups (N = 5/treatment) for an aversion trial. Treatment lambs received the ingenol in ethanol and control lambs received only ethanol. The lambs were about 5 months old.

During the seven-day experiment, except for the brief training or trial periods, lambs were held together in an outdoor pen and had ad libitum access to alfalfa (*Medicago sativa*) hay and salt from 0800 to 1700 hr and had continuous access to water. During a five-day pretrial period, sheep were offered alfalfa pellets from 0800 to 0830 hr in individual outdoor pens to accustom them to the trial procedure.

On the day of the trial, each sheep was offered 300 g of a novel food (milo) in the individual pens from 0800 to 0830 hr. At 0830 hr, after they consumed the novel feed, they were released to their outdoor pen.

Sheep were given jugular injections of ingenol in ethanol (1 mg ingenol 3,20-dibenzoate/ml ethanol) or ethanol alone from 0915 to 0945 hr and from 1015 to 1045 hr. The dosage of ingenol was determined from the concentration of ingenol 3,20-dibenzoate in air-dried leafy spurge (0.0002%) (Kupchan et al., 1976) and a dosage of air-dried leafy spurge that has caused marked elevations of blood cortisol in sheep (0.3% of body weight) indicating physiological stress (Kronberg et al., 1993c) (twice the dosage of air-dried material necessary to cause an aversive response from leafy spurge; Kronberg et al., 1993a). Ingenol 3,20-dibenzoate was purchased from LC Services Corp., Woburn, Massachusetts (Lot BG-101). Each lamb received a dose of 0.6% of body weight (BW) of the appropriate solution at each dosing. On the second day of the trial, each lamb was offered 300 g of novel feed from 0800 to 0830 hr. The amount of novel feed consumed by each animal was recorded daily.

Experiment 2. This experiment studied the effect of fermenting leafy spurge with ruminal digesta from sheep or goats on the subsequent aversiveness of leafy spurge to sheep. Ten ruminally fistulated white-faced lambs (mean wt, 49 ± 11 kg) were randomly divided into two treatment groups (N = 5/treatment) for an aversion trial. The lambs were about 11 months old and were different animals from those used in the first experiment.

Sheep were held in an outdoor pen and had ad libitum access to alfalfa hay and salt from 0700 to 1700 hr and had continuous access to water. During a four-day pretrial period, the sheep were offered alfalfa pellets from 0700 to 0730 hr in individual indoor pens to accustom them to the trial procedure.

On each day of the five-day trial, each sheep was offered 300 g of a novel food (rolled oats) in the individual pens from 0700 to 0730 hr. On the third and fourth days of the trial, after the 30-min period of novel food consumption, sheep were dosed, via their ruminal fistula, with leafy spurge that had been fermented with either sheep or goat digesta for 12.5 hr. The amount of novel food consumed by each animal was recorded daily.

Leafy spurge/digesta fermentations were conducted with the following procedure. For each day that fermented mixtures were dosed, freeze-dried, and ground (1-mm screen) leafy spurge equivalent to 0.10% of body weight (ca. 49 g/animal) was placed into 1-liter Erlenmeyer flasks. Buffer solution was added to each flask and mixed with the leafy spurge, then flasks were placed in an incubator (39°C) to warm. Ruminal digesta from two to four donors of each species was collected into prewarmed insulated containers by species and mixed. Donor animals were maintained on alfalfa. Digesta was added to each flask within a half hour of its collection. Flasks were incubated for 12.5 hr and agitated 3.5 hr after the start of the fermentations. Fermentations were stopped by placing the flasks in ice water. The buffer recipe and proportions of leafy spurge, buffer, and ruminal digesta followed the Barnes modification of the Tilley and Terry in vitro technique (Harris, 1970) with the exception that whole ruminal digesta was used instead of ruminal liquor because the majority of ruminal bacteria are associated with the particulate phase (Forsberg and Lam, 1977; Craig et al., 1987).

Data Analysis. For both experiments, novel food intake after dosing was the dependent variable. Data for experiment 1 were analyzed by analysis of variance, and data for experiment 2 were analyzed by repeated-measures analysis of covariance (SAS, 1986). Novel food intake on the day leafy spurge was first dosed was the covariate, treatment group was the between-animal effect, and day postdose was the within-animal effect.

RESULTS

Experiment 1. The treatment and control groups had similar intakes of novel food on the first day (P = 0.21; 222 and 273 g, respectively) and on the second day (P = 0.34; 300 and 277 g, respectively). Neither group displayed an aversive response to the novel food on day 2 of the trial.

Experiment 2. Sheep dosed with leafy spurge fermented with sheep digesta consumed less novel feed than sheep that received the goat digesta treatment ($P = 0.03$; 122 compared to 203 g, respectively). The aversive effect of spurge fermented with sheep digesta tended to increase after the second dosing with this mixture (treatment × day interaction, $P = 0.16$; Figure 1). The intake of NF on day 3 before dosing (the covariate) accounted for a significant amount of variation ($P = 0.005$) in postdose NF intake.

DISCUSSION

Results from the first experiment suggest that either ingenol 3,20-dibenzoate is not an aversive chemical in leafy spurge or it does not stimulate the development of a conditioned flavor aversion by reaching the area postrema in the blood. Conditioned flavor aversions can also be instigated by stimulation of visceral afferent nerves (Willems and Lefebvre, 1986). Therefore, this ingenol may stimulate flavor aversion development via another route, and this possibility should be investigated. Alternatively, one of the other ingenols found in leafy spurge may be an aversive compound.

Results from experiment 2 suggest that fermenting leafy spurge with goat ruminal digesta may limit its capacity to elicit a conditioned flavor aversion in

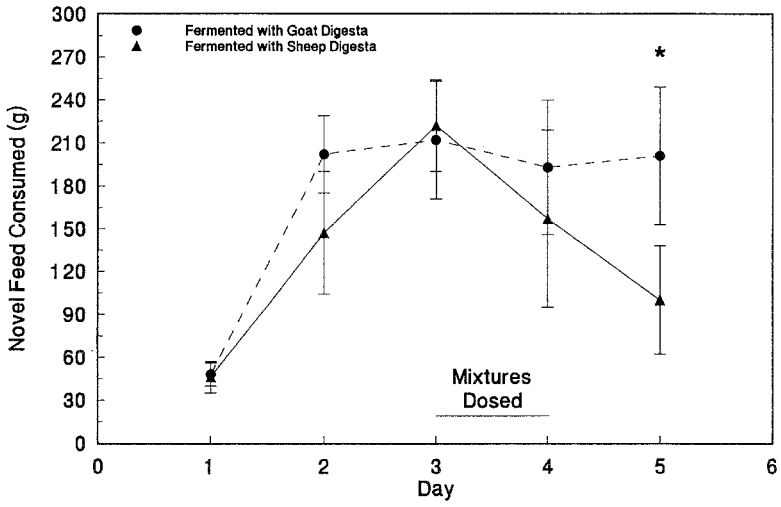

FIG. 1. Consumption of novel feed (g) during a 0.5-hr period by two treatment groups of sheep on each day of a five-day trial. Group 1 and 2 sheep received leafy spurge fermented with ruminal digesta from sheep and goats, respectively. The spurge/digesta mixtures were dosed on days 3 and 4; *indicates that treatment groups differed ($P < 0.05$) in intake of novel feed on that day. Bars represent ± SE.

sheep. In contrast, fermenting leafy spurge with sheep ruminal digesta either (1) does not nullify its capacity to elicit an aversive response from sheep, or (2) creates a by-product during leafy spurge fermentation that is aversive. However, if the second alternative occurs, the spurge/goat-digesta mixture might still have elicited an aversion in sheep.

It is well established that ruminal microorganisms can detoxify various phytotoxins (James et al., 1975; Allison et al., 1990; Craig et al., 1992; Duncan and Milne, 1992), and ruminal microbes can adapt and increase their capacity for detoxifying phytochemicals by altering their number or metabolic capacity or by altering the composition of the microbial community (Lindroth, 1988). However, ruminal microbes in ruminant individuals and species apparently vary in their capacity to degrade particular toxins. Wachenheim et al. (1992) have shown that the ruminal bacteria that metabolize pyrrolizidine alkaloids exist in cattle, sheep, and goats, but only sheep and goats have populations of these bacteria that are large enough to degrade the toxic alkaloids in a timely manner.

Hungate (1966) noted that various ruminal microbes such as *Veillonella alcalescens*, Quin's oval and large selemonads are much more abundant in sheep than in cattle. Several variables may account for differences in composition of rumen microbial communities and fermentation patterns among ruminant species and among individuals of the same species (Hungate, 1966). These include: ruminal pH, osmolarity and redox potential, nutrient availability and recycling to the rumen (via saliva and blood), dietary intake rate and composition, saliva production, and digesta particle size and passage rate (therefore, degree of rumination is indirectly influential) (Hungate, 1966; Owens and Goetsch, 1988; Yokoyama and Johnson, 1988).

Ruminal microbes can be either highly specialized, intermediate, or general in the type of nutrients that they metabolize; consequently, variations in dietary composition among ruminant species is reflected in the variation in rumen microbial compositions (Yokoyama and Johnson, 1988). Ruminal bacteria vary widely in the substrates they ferment (Russell, 1984). For example, *Bacteroides succinogenes*, *Ruminococcus flavefaciens*, and *R. albus* require ammonia, whereas *Selenomonas ruminantium* and *Peptostreptoccus elsdenii* apparently do not (Hungate, 1966). Additionally, the vitamins biotin, folic acid, thiamine, pyridoxine, and pantothenic acid are required by certain species, but not others (Hungate, 1966). Rumen pH, one of the most variable environmental factors, can greatly affect the microbial population (Hungate, 1966; Yokoyama and Johnson, 1988). Reduced feed particle size increases direct contact between bacteria and substrate; consequently, the rate of bacterial adhesion to feed particles and, therefore, fermentation rate can be influenced by the degree of mastication (Cheng, 1987).

There is limited information on variability of factors that could account for variation in rumen microbial communities among ruminant species. Hoppe et

al. (1977) collected blood and ruminal samples from freshly killed Maasai sheep and goats, Thomson's and Grant's gazelles (*Gazella thomsoni* and *G. granti*, respectively), and impalas (*Aepycerus melampus*) during a dry season. Ruminal pH for these species was: 6.12, 6.10, 6.04, 6.01, and 6.30, respectively, and ruminal ammonia nitrogen concentrations were: 13.8, 16.6, 18.5, 14.1, and 21.1 mg/100 ml, respectively. Thornton (1970) fed cattle and sheep the same amount (metabolic BW basis) of the same diet and observed that cattle had slightly lower plasma urea nitrogen (PUN) and ruminal fluid ammonia nitrogen concentrations (1.49 and 0.70 mg/100 ml, respectively) than sheep (1.92 and 0.97 mg/100 ml, respectively). When cattle (crossbreds of *Bos tarus* and *B. indicus*) and sheep were fed the tropical forage spear grass (*Heteropogon contortus*) ad libitum, cattle and sheep had daily nitrogen intakes of 0.09 and 0.08 g/kg of BW/day, respectively, and PUN levels of 5.6 and 14.5 mg/100 ml, respectively (Siebert and Kennedy, 1972). When they were fed a diet of 80% spear grass and 20% alfalfa, cattle and sheep had daily nitrogen intakes of 0.27 and 0.24 g/kg of BW/day, respectively, yet cattle still had lower PUN levels than sheep (12.1 and 19.5 mg/100 ml, respectively) (Seibert and Kennedy, 1972).

Plasma urea nitrogen was linearly related to salivary urea concentration in cattle (Bailey and Balch, 1961) and sheep (Somers, 1961) up to a certain concentration of PUN, and the diffusion of urea from the blood across the rumen epithelium (urea is hydrolyzed to ammonia as it diffuses through the epithelium) was linearly related to blood urea concentration (Houpt and Houpt, 1968). Therefore, it is logical to assume that lower levels of urea were entering the rumens of cattle than of sheep in Thornton's (1970) and Seibert and Kennedy's (1972) studies, and this difference probably influenced the composition of their rumen microbial communities.

Huston et al. (1986) fed forage sorghum (*Sorghum* spp.) hay to sheep, goats, and white-tailed deer (*Odocoileus virginianus*). The digestibility of sorghum hay was different among sheep, goats, and deer (56.4, 61.5, and 51.8%, respectively) as was mean retention time of sorghum particles in the gastrointestinal tract (35.5, 59.6, and 33.0 hr, respectively). Reid et al. (1990) compared the utilization of warm- and cool-season forage hays by cattle, sheep, and goats and observed that: (1) cattle generally had higher intakes than sheep and goats, and (2) sheep and goats had faster particle passage rates than cattle. Playne (1978) fed cattle and sheep similar amounts (BW basis) of low-quality tropical grass hay and observed that the digestibility of this hay was much higher in cattle than in sheep (49.6 vs 34.6%, respectively) and that cattle had higher serum SO_4-S concentrations and had a better sulfur balance than sheep did. Playne (1978) concluded that this difference was not due to the diet selected by the two species, but was a result of greater neutral detergent fiber (NDF) digestion by the cattle, and suggested that differential NDF digestion may result from

differences between cattle and sheep in their ability to recycle nutrients to their rumen (e.g., sulfur) and, consequently, differences in rumen microbial activity. These reports support our contention that there is variation in ruminal environments and probably differences in rumen microbial communities among ruminant species. This variability may account for some of the diet selection differences among these species.

Results from our second experiment may explain why goats generally graze leafy spurge more readily than do sheep. If the level of aversive postingestive feedback derived from a food is low and/or delayed and its nutritional value (positive feedback) is high and/or promptly experienced, ruminants appear to have more attraction than aversion to it and increase their intake of the food (Launchbaugh and Provenza, 1992; Provenza and Cincotta, 1992). Leafy spurge has high crude protein and digestible energy levels similar to alfalfa (Fox et al., 1991). We speculate that leafy spurge offers more positive than negative stimuli for goats because of its treatment in the goat's rumen; therefore, they continue to consume it. In contrast, leafy spurge causes more negative than positive stimuli for sheep because of their ruminal treatment of spurge; therefore, they do not continue to consume it. Provenza et al. (1992) offered a similar explanation for observations of cattle either grazing or avoiding tall larkspur (*Delphinium barbeyi*).

We interpret our findings to suggest that differences among livestock species in selection for leafy spurge may be caused by processes occurring in the rumen. We believe that this report offers the first evidence that differential activity in the rumen of two ruminant species may account for differences in their diet selection. However, it is also possible that degradation of spurge chemicals in the goat occurs within its own tissues (e.g., its liver). The goal of our research is to discover the mechanisms that allow goats to consume leafy spurge with apparent impunity and to use this knowledge to bestow similar abilities to other species of livestock.

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