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ECOLOGICAL IMPLICATIONS OF FLAVOR GENERALIZATION BY SHEEP:

ROLE OF FLAVOR INTENSITY AND VARIATION IN TOXIN DOSE

by

Karen L. Launchbaugh

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Range Science

UTAH STATE UNIVERSITY
Logan, Utah

1992

DEDICATION

In memory of Dr. David F. Balph.

My great teacher, mentor, and friend.

ACKNOWLEDGEMENTS

I rightfully begin this dissertation with thanks to the people who made it possible. My sincerest thanks to my major professor Fred Provenza for his careful guidance and friendship in my Ph.D. program. I am indebted to him for helping me develop not only the skills for research and teaching but also a philosophy to accompany them. I would also like to thank the members of my graduate committee, Carl Cheney, John Malechek, James Pfister, and John Walker, for their encouragement, constructive criticism, and active participation in my research. I am grateful to Philip Urness who reviewed the latter copies of this dissertation, participated in my dissertation defense, and provided many stimulating intellectual conversations during my Ph.D. program.

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No words can adequately express my appreciation to my husband Karl Launchbaugh. His encouragement, care, and patience contributed to every aspect of this research.

Karen Launchbaugh

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ABSTRACT

Ecological Implications of Flavor Generalization by Sheep:

Role of Flavor Intensity and Variation in Toxin Dose

by

Karen L. Launchbaugh, Doctor of Philosophy

Utah State University, 1992

Major Professor: Dr. Frederick D. Provenza

Department: Range Science

Researchers studying diet selection of ungulate herbivores have generally considered plant palatability independent of animals' dietary history. However, more recent studies demonstrate that experiences within the life of an animal strongly influence plant selection. We are beginning to understand how food preferences and aversions are formed through gastro-intestinal feedback. My research specifically examines factors that influence the formation of conditioned flavor aversions in the generalist herbivore, sheep.

I first examined how variability of food toxicity affects the intake of those foods. I determined that sheep apparently have several mechanisms for regulating intake of toxic foods regardless of whether or not toxic variation can be detected through

flavor changes. When changes in flavor correspond to changes in toxicity, animals adjusted intake based on an increase or decrease in toxin concentration. When toxic variation was not detectable through flavor, animals adopted a conservative strategy of eating an amount based on the maximum toxin dose they had experienced.

I was also interested in how illness following the consumption of one food influences the selection of other foods. In diet selection, animals may generalize selection responses among foods with similar flavors. Generalization may be particularly important in the selection of novel foods, i.e., a new food may look, smell, or taste like a familiar food that is preferred or avoided. In several experiments on the generalization of flavor aversions I found that: 1) sheep generalize aversions from familiar to novel foods when both foods had a flavor in common; 2) the more sick an animal got after eating a food the greater the aversion formed to the food and the greater the generalization of that aversion to new foods; 3) the salience or intensity of flavor did not affect the strength of conditioned flavor aversions in sheep on the generalization of the aversion, but this may not always be the case; 4) flavor intensity strongly influenced the acceptance of a novel food. A novel food (wheat) with a strong flavor (3% added ground oregano) was more avoided than a novel food (wheat) with a mild flavor (1% oregano added).

CHAPTER I

INTRODUCTION

Approaches to Diet Selection Research

Selective grazing is a reciprocal process that, on one hand, determines the nutritional welfare of the herbivore, and on the other hand, alters the dynamics of the plant community. The nutrient status of free-grazing animals is obviously a direct consequence of diet selection. Animals generally select diets higher in digestible nitrogen and energy and lower in fiber and phytotoxins than available herbage (Arnold and Dudzinski, 1978). However, diet selection mechanisms are not infallible as evidenced by livestock poisonings. Selective grazing is also a major cause of range retrogression in many ecosystems. The selection of some plants and avoidance of others leads to a competitive disadvantage for the preferred relative to the avoided species. Most grazing management systems are designed to ameliorate this adverse effect of diet selection. These important impacts of selective grazing have fueled research on diet selection.

One approach to studying the impacts of selective grazing has been to attempt to predict animals' diets; if diets could be adequately predicted, then grazing impacts could be anticipated and managed. Predictive models based on preference ratings (Hurd and Pond, 1958; Nelson, 1978) and forage correlation with nutritional quality

(Ellis et al., 1976; Sibbald et al., 1979) have been applied in rangeland and pasture settings. Optimal foraging theory has also produced several predictive foraging models (Schluter, 1981; Owen-Smith and Novellie, 1982). These models have been successful in predicting gross diet selection patterns and have described the consistency with which particular plants are eaten. But, the results are generally site specific and do not lead to the type of detailed predictions that are needed for more accurate plant management practices or projections of animal nutrient status.

Another approach to understanding diet selection has been to study "why" animals select particular foods. Initial theories assumed that diet selection was a genetically fixed process (i.e., hedyphagia, euphagia; Provenza and Balph, 1990). However, more recent studies demonstrate that experiences within the life of an animal strongly influence plant selection (Provenza and Balph 1987; 1988). Although an understanding of the mechanisms of diet selection has not led to accurate predictive models, it has produced opportunities to control and manage selective grazing through diet training procedures (Provenza and Balph, 1987).

We are beginning to understand how food preferences and aversions are formed based on gastro-intestinal feedback (Garcia, 1989; Provenza et al., 1992a). Many specific properties of conditioned preferences and aversions first discovered in work on laboratory animals have been extended to domestic livestock; examples include: long-delay learning (Burritt and Provenza, 1992a), the tendency of herbivores to associate illness with novel rather than familiar foods (Burritt and Provenza, 1989; 1992a), conditioned preferences for flavors associated with energy sources (Burritt and

Provenza, 1992b), and the formation of aversions in anesthetized animals (Provenza et al., 1992b). Social models have also been shown to strongly influence plant selection (Thorhallsdottir, 1988) and avoidance (Mirza, 1991).

Specific Goals of My Research

My research specifically examines factors that influence the formation of conditioned flavor aversions (CFAs) in the generalist herbivore, sheep. Understanding food aversions is important for diet training management (Provenza and Balph, 1987), for mediating animal damage (Gustavson et al., 1982; Nicolaus, 1987), and for understanding animal response to poisonous plants (Provenza et al., 1988; 1992a).

The second chapter of this dissertation summarizes three experiments that examined how changes in food toxicity affected the intake of food by sheep. In the first experiment I repeatedly exposed lambs to oats containing a specified concentration of the gastro-intestinal toxin, lithium chloride (LiCl). Increases or decreases in the concentration of LiCl were then related to changes in food intake. This is analogous to foraging situations where changes in the concentration of a plant toxin are associated with changes in plant flavor. The second experiment examined how flavor aversions were formed when animals sampled foods with varying concentrations of the same flavor before they experienced illness. The experiment related to how animals might learn to differentially select between plant parts that vary in phytotoxin concentration. The third experiment monitored intake of oregano-flavored barley followed by a variable dose of LiCl. This experiment was analogous to a foraging situation where changes in

plant toxicity are not associated with plant flavor. The results of these three experiments are important in understanding how animals respond to plants that vary in toxicity depending on location, time of year, weather conditions, or animal characteristics.

Most research on CFAs has taken a one-food-at-a-time approach. Thus, considerable knowledge exists on how illness following the consumption of a food affects the palatability of that food. However, very little is known about how illness following the consumption of one food influences the selection of other foods. Chapters three and four of this dissertation focus on factors that not only influence the formation of a CFA but also the generalization of that CFA to other foods.

Generalization occurs when animals respond in a uniform way to a group of stimuli (Mazur, 1990). In diet selection, animals may generalize selection responses among foods with similar flavors. For example, a group of poisonous plants may contain a similar toxin and, therefore, a common salient flavor; herbivores may avoid a group of plants based on a common flavor. Generalization may also be important in the selection of novel foods; a new food may look, smell, or taste like a familiar food that is preferred or avoided. In this way, generalized aversions or preferences may influence the ingestion of new foods (Tapper and Halpern, 1968; Smith and Theodore, 1984; Nicolaus, 1987).

The third chapter reports a study in which I asked if illness following the consumption of one food influenced the intake of another food with a similar flavor. In this way, I examined the generalization of a CFA based on a common flavor. The

generalization of a CFA based on odor alone was also assessed. The results of these experiments are discussed in reference to the potential effectiveness of flavor mimicry as an anti-herbivore mechanism in plants.

Experiments reported in the fourth chapter examined the influence of flavor intensity and toxin dose on the formation of a CFA, the generalization of that CFA to another food with a similar flavor, and the acceptance of a novel food. The results of these experiments are examined in relation to animals' responses to new foods and preparing animals for new foraging environments.

In the synthesis, chapter 5, I summarize all my research results. I then discuss the results in relation to plant defense theories that are based on assumptions of how chemical components in plants deter grazing. My results suggest that while some of these assumptions are valid, others are questionable. Finally, I suggest several plant strategies that may effectively deter grazing by mammalian herbivores.

Literature Cited

- Arnold, G.W. and M.L. Dudzinski. 1978. *Ethology of Free-Ranging Domestic Animals*. Elsevier Sci. Pub. Co., Amsterdam.
- Burritt, E.A. and F.D. Provenza. 1989. Food aversion learning: ability of lambs to distinguish safe from harmful foods. *J. Anim. Sci.* 67:1732.
- Burritt, E.A. and F.D. Provenza. 1992a. Ability of lambs to learn with a delay between food ingestion and consequences given meals containing novel and familiar foods. *Appl. Anim. Behav. Sci.* (In press).
- Burritt, E.A. and F.D. Provenza. 1992b. Lambs form preferences for non-nutritive flavors paired with glucose. *J. Anim. Sci.* (In press).
- Ellis, J.E., J.A. Wiens, C.F. Rodell and J.C. Anway. 1976. A conceptual model of diet selection as an ecosystem process. *J. Theor. Biol.* 60:93.
- Garcia, J. 1989. Food for Tolman: cognition and cathexis in concert. In: T. Archer and L. Nilsson (Ed.) *Aversions, Avoidance and Anxiety*. pp 45-85. Lawrence Erlbaum Assoc., Hillsdale, NJ.
- Gustavson, C.R., J.R. Jowsey and D.N. Milligan. 1982. A 3-year evaluation of taste aversion coyote control in Saskatchewan. *J. Range. Manage.* 35:57.
- Hurd, R.M. and F.W. Pond. 1958. Relative preference and productivity of species on summer cattle ranges, Big Horn Mountains, Wyoming. *J. Range Manage.* 11:109.
- Mazur, J.E. 1990. *Learning and Behavior*. Prentice-Hall Inc., Englewood Cliffs, NJ.
- Mirza, S. 1991. Mother's role in socially induced food avoidance in lambs. Ph.D. Dissertation. Utah State Univ., Logan.
- Nelson, J.R. 1978. Maximizing mixed animal species stocking rates under proper-use management. *J. Wildl. Manage.* 42:172.
- Nicolaus, L.K. 1987. Conditioned aversions in a guild of egg predators: implications for aposematism and prey defense mimicry. *Am. Midl. Nat.* 117:405.
- Provenza, F.D. and D.F. Balph. 1987. Diet learning by domestic ruminants: theory, evidence and practical implications. *Appl. Anim. Behav. Sci.* 18:211.

- Provenza, F.D. and D.F. Balph. 1988. The development of dietary choice in livestock on rangelands and its implications for management. *J. Anim. Sci.* 66:2356.
- Provenza, F.D. and D.F. Balph. 1990. Applicability of five diet-selection models to various foraging challenges ruminants encounter. In: R.N. Hughes (Ed.) *Behavioral Mechanisms of Food Selection*. pp 449-460. Springer-Verlag, Berlin.
- Provenza, F.D., D.F. Balph, D.D. Dwyer, M.H. Ralphs and J.A. Pfister. 1988. Toward understanding the behavioral responses of livestock to poisonous plants. In: L.F. James, M.H. Ralphs, and D.B. Nielsen (Ed.) *The Ecology and Economic Impact of Poisonous Plants on Livestock Production*. pp 407-425. Westview Press, Boulder, CO.
- Provenza, F.D., J.A. Pfister and C.D. Cheney. 1992a. Mechanisms of learning in diet selection with reference to phytotoxicosis in herbivores. *J. Range Manage.* 45:36.
- Provenza, F.D., J.J. Lynch and J.V. Nolan. 1992b. Food aversion conditioned in anesthetized sheep. *J. Comp. Physiol. Psych.* (submitted).
- Owen-Smith, N. and P. Novellie. 1982. What should a clever ungulate eat? *Am. Nat.* 118:151.
- Schluter, D. 1981. Does the theory of optimal diets apply in complex environments? *Am. Nat.* 118:139.
- Sibbald, A.R., T.J. Maxwell and J. Eadie. 1979. A conceptual approach to the modelling of herbage intake by hill sheep. *Agr. Sys.* 4:119.
- Smith, D.V. and R.M. Theodore. 1984. Conditioned taste aversions: generalization to taste mixtures. *Physiol. & Behav.* 32:983.
- Tapper, D.N. and B.P. Halpern. 1968. Taste stimuli: a behavioral categorization. *Science* 161:708.
- Thorhallsdottir, A.G. 1988. The role of social models in the development of food preferences of lambs. Ph.D. Dissertation. Utah State Univ., Logan.

CHAPTER II

HOW HERBIVORES TRACK VARIABLE ENVIRONMENTS: RESPONSE TO VARIABILITY OF PHYTOTOXINS

ABSTRACT - The concentration of toxins in plants can vary depending on plant phenology and environmental factors. Changes in toxicity may or may not be associated with changes in flavor. Thus, one of the greatest foraging challenges facing herbivores is tracking changes in plant toxicity. In experiment 1, I asked, if animals have experience with a tastable toxin at one concentration, do they adjust intake when the concentration of the toxin changes? Sixty lambs were divided into 4 groups and offered oats containing either 0% (control), 0.75% (low), 1.5% (medium), or 2.25% (high) lithium chloride (LiCl). After 10 days, all lambs were offered oats with 1.5% LiCl. Lambs in the low treatment decreased intake while the lambs in the high treatment increased intake when offered oats with 1.5% LiCl.

In experiment 2, I asked if sheep sample foods with varying intensities of the same flavor, and then experience gastro-intestinal illness, do they then avoid foods with greater concentrations of the flavor? Lambs were offered barley with a high and low concentration of either sodium saccharin (a sweet flavor) or aluminum sulfate (a bitter flavor). Each lamb initially ate about the same amount of barley with the low and high flavor concentrations. Then, lambs received a low dose of LiCl in a gelatin capsule. The

next day, lambs were again offered barley with a high and low concentration of either saccharin or alum. Lambs preferred the barley with the low flavor level.

Experiment 3 examined the effect of variable toxicity on intake when flavor remained constant. Lambs were offered oregano-flavored barley followed by a dose of LiCl for 10 days. The LiCl dose was either 1.5% of the amount of barley eaten (medium control), 2.25% of the amount eaten (high control), or a randomly selected dose of 0.75, 1.5 or 2.25% of the amount eaten (variable treatment). Lambs in the medium control ate more oregano-barley in the last 6 days of the trial than the high control or variable treatment groups which were not significantly different from each other.

INTRODUCTION

Herbivores face many challenges in selecting a diet to meet their nutritional needs. They must overcome chemical plant defenses and select among plants that vary in nutrient value and toxicity in time and space (Provenza and Balph, 1990; Laca and Demment, 1991). For instance, plant toxicity depends on environmental conditions such as drought (Harborne, 1991), soil nutrient levels, and shade (Bryant et al., 1992). Toxicity and nutrient value also vary with plant phenology, growth stage, and plant part (McKey, 1979; Bryant et al., 1992), and in response to herbivory or mechanical damage (Harborne, 1991).

The effect of a phytotoxin on an herbivore clearly depends on the rate and amount of toxin consumed, but other factors are also important. For instance, diet composition may be important because different phytochemicals can interact in the gut to either

decrease or increase their toxic effect (Freeland and Janzen, 1974). Conditions in the gut, such as pH, temperature, and solution polarity can alter the chemical reactivity and, therefore, toxicity of secondary compounds (McArthur et al., 1991). Toxicity may also depend on animal age, sex, and hormonal or nutritional state (Freeland and Janzen, 1974; Brattsten, 1979). Finally, toxicity can be altered by animal experience. After sustained intake of a particular compound an animal may exhibit an increased ability to detoxify the chemical. For example, some mammals exhibit increased production of proline-rich salivary proteins in response to diets high in tannins (Robbins et al., 1987).

Many authors have recognized that plant toxicity varies, depending on plant and animal characteristics, but few authors have postulated means by which herbivores avoid lethal doses of phytotoxins in variable environments. Selecting a diverse diet has been suggested as a way that herbivores may limit exposure to any single phytotoxin (Freeland and Janzen, 1974; Laycock et al., 1988). Flavor-consequence relationships, based on post-ingestive feedback, have also been suggested as a way that herbivores recognize changes in toxicity (Provenza et al., 1988; 1992a). Neither of these mechanisms has been rigorously tested. The purposes of the following three experiments were to examine the ability of a generalist herbivore (sheep) to adjust intake when (1) toxicity changes were associated with flavor changes, (2) foods with varying flavor concentration were sampled prior to illness, and (3) flavor was not an indicator of toxicity.

EXPERIMENT 1

Some researchers argue that animals identify plant toxins by associating food flavor (odor and taste) with post-ingestive feedback (Garcia, 1989; Provenza and Balph, 1990). Many plant allelochemicals have salient flavors that may impart a distinct flavor to plants containing those chemicals. For instance, some tannins presumably are astringent to herbivores, and some flavanones and alkaloids presumably taste bitter to herbivores (Laycock et al., 1988; Harborne, 1991). When flavor and toxicity are highly correlated, herbivores might first learn to regulate food intake from post-ingestive feedback, and then simply adjust intake on the basis of flavor concentration.

My first experiment examined the generalization of a conditioned flavor aversion (CFA) across different concentrations of the same flavor. I asked, if animals have experience with a tastable toxin at one concentration, do they adjust intake when the concentration of the toxin changes?

Materials and Methods. Sixty orphan lambs that were crosses of Columbia, Rambouillet, Suffolk, Targhee, and Finnish Landrace breeds were used to conduct this experiment. The lambs were raised to 6 months age were penned individually for 10 days and offered pelletized alfalfa (*Medicago sativa*) *ad libitum* for 1 hour every morning. They had access to water and salt at all times and pens were set side-by-side to allow social contact between lambs. For 30 minutes in the afternoon of each day during the 10-day conditioning period, each lamb was offered 500 g of a novel food, oats, containing none or various levels of lithium chloride (LiCl).

The lambs were assigned at random to 1 of 4 treatments: (1) control, oats (*Avena sativa*) with no LiCl, (2) low, oats with 0.75% LiCl, (3) medium, oats with 1.5% LiCl, or (4) high, oats with 2.25% LiCl. LiCl was applied to the oats by dissolving it in water (a saturated solution) and then mixing it thoroughly with oats and then drying the LiCl-oats mixture at 60°C for 12 hours. Daily consumption of LiCl-oats was recorded.

The test consisted of offering all lambs 500 g of oats with 1.5% LiCl (the medium treatment level) for 30 minutes on the first afternoon following conditioning (day 11 of the trial). On the test day, lambs were offered alfalfa pellets *ad libitum* in the same manner as during conditioning. The testing procedure was repeated on days 12, 13, and 14 as a follow-up period to monitor subsequent changes in intake.

The treatments were compared statistically with an analysis of variance (ANOVA) on the first day of conditioning to examine differences in initial intake. Data were also analyzed with a repeated measures ANOVA to examine intake differences throughout the conditioning and follow-up periods with day as the repeated measure. Intake on the test day was compared with a separate ANOVA. Variables compared were intake of LiCl-oats and amount of LiCl consumed. Fisher's LSD procedure was used to separate treatment means. A paired t-test was employed to compare intake on the last day of conditioning (day 10) with that on the test day (day 11).

Results. On the first day of conditioning, there was no difference in intake between the control, low, and high treatments. However, lambs in the medium treatment ate less than other lambs ($P < 0.05$) for no obvious reason (Figure 1). In the remaining days of conditioning, treatment patterns emerged so that by day 10, the concentration of LiCl

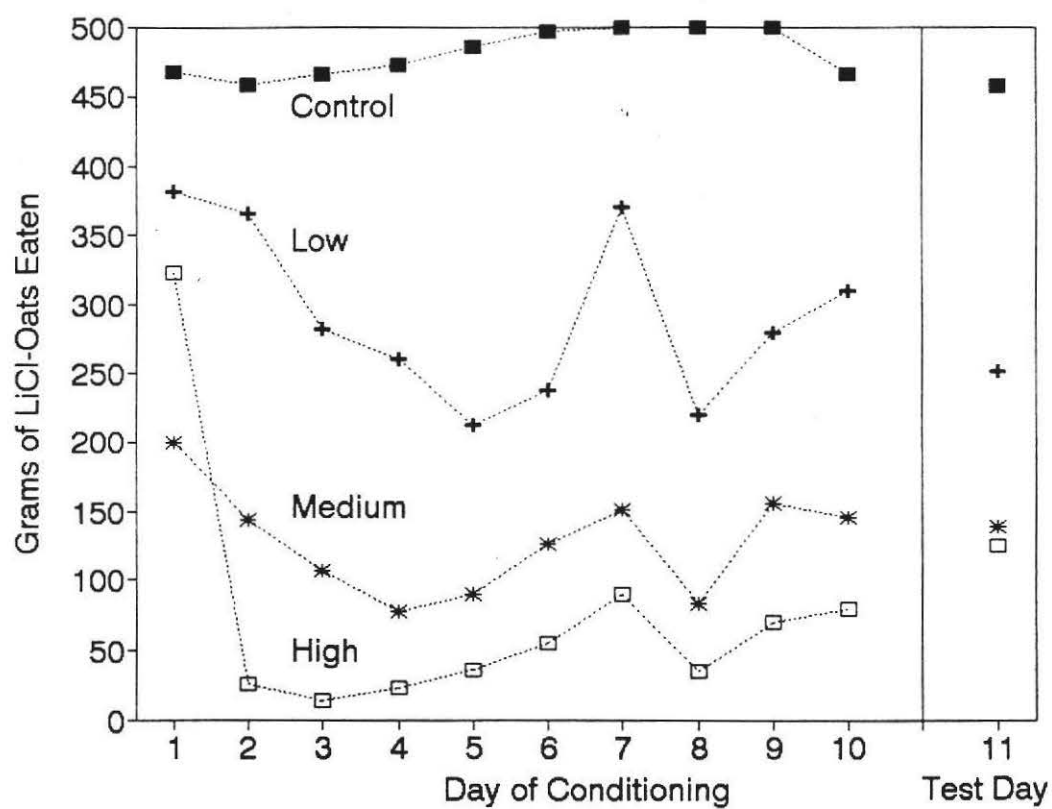


Fig. 1. Daily intake of oats by lambs in the control and LiCl-oats by lambs in the low, medium, and high treatments during the conditioning period, and intake of LiCl-oats by all lambs on the test day of experiment 1.

on oats had a strong effect on intake ($P < 0.001$; Figure 1). The control group consumed the most oats, while lambs that received low, medium, and high concentrations of LiCl on oats ate less, respectively (Figure 1). Though consumption of LiCl-oats differed by treatment (excluding the control), the absolute amount of LiCl consumed on day 10 was the same for all treatments (Table 1).

Table 1. Amount of LiCl-Oats and LiCl ingested by lambs on day 10 of the conditioning period of experiment 1.

Treatment	LiCl-Oats Eaten (g \pm SE)	LiCl Ingested (mg/kg \pm SE)
Low	310.0 ^a \pm 25.6	68.5 ^a \pm 5.1
Medium	145.6 ^b \pm 18.6	66.6 ^a \pm 6.9
High	79.5 ^c \pm 17.3	52.5 ^a \pm 11.1

^{abc}Means for treatments followed by a different letter are different ($P < 0.05$).

On the test day, when all lambs were offered oats with 1.5% LiCl, lambs in the medium and control groups ate similar amounts as they had on the last day of conditioning ($P < 0.05$). However, lambs in the high treatment increased intake, while lambs in the low treatment decreased intake compared with the last day of conditioning ($P < 0.05$).

During the follow-up period (days 12, 13, and 14) overall intake differed by treatment group ($P < 0.002$) with lambs in the medium and low treatments eating more than lambs in the control or high treatments (Figure 2). The reason for these intake differences are not apparent. By day 14, treatment differences had disappeared

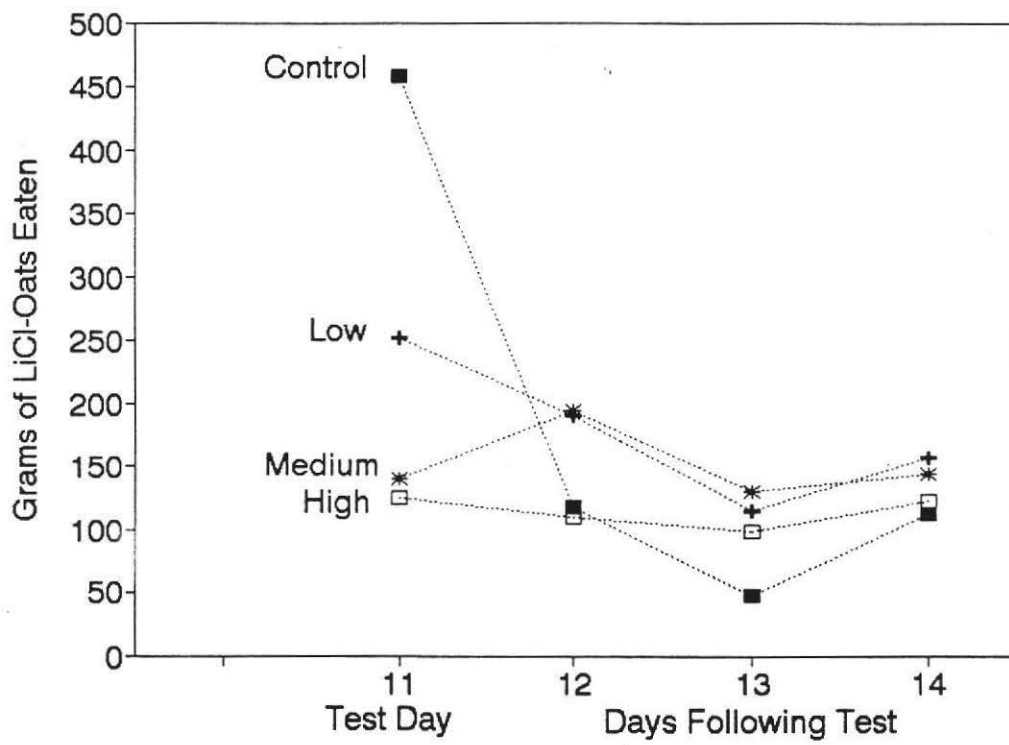


Fig. 2. Intake of LiCl-oats by lambs on the test day of experiment 1 and the three days following the test.

($P = 3.373$). Intake varied by day ($P < 0.002$) but there was no interaction between day and treatment ($P = 0.457$).

Discussion. Lambs adjusted their intake of LiCl-oats in response to a change in LiCl concentration, even though their experience was limited to a single concentration. These results are consistent with research conducted on rat subjects to examine the generalization of a flavor aversion across flavor concentration gradients (Nowlis, 1974; Scott and Giza, 1987; Spector and Grill, 1988). It is possible that these results were influenced by gastro-intestinal feedback experienced during the 30-minute exposure period (Provenza et al. 1992b). However, since the addition of LiCl to oats on the test day did not influence intake for the control group it is likely the feedback during the exposure period did not significantly influence the results. This apparent lack of association between intake and toxin dose during the test period may be explained by the field observation that lambs generally completed their feeding bout in 10-12 minutes even though they were allowed 30 minutes to eat LiCl-oats.

These results suggest that sheep could adjust their intake of phytotoxins based on flavor alone, provided the concentration of the toxin was highly correlated with the plant flavor. A strong relationship between toxin and flavor is likely to occur when a plant is defended by a single or few phytochemicals that have a detectable flavor. Ecological studies that reveal a correlation between the concentration of a toxin and palatability may be examples of the above relationship. For example, the amount of phenolic resin on the leaves and stems of creosote bush (*Larrea tridentata*) is directly related to herbivore acceptance (Meyer and Karasov, 1991). The quantity of phenolic resin even

influences palatability when extracted from creosote foliage and placed on a pelleted feed.

Note that lambs limited intake of LiCl to an average 62.7 mg/kg (SE=4.5) regardless of the concentration of LiCl on the food. This agrees with the common observation that animals do not completely avoid most toxic foods, but continue to eat the plants and maintain intake below some toxic threshold (Freeland and Janzen, 1974). Burritt and Provenza (1989) reported voluntary intake of LiCl by sheep as 27 mg/kg when LiCl was placed on oats at a 2% concentration. This is half the level recorded in the present study. The difference may be explained by differences in how LiCl-oats was offered to lambs. I presented LiCl-oats separately from the basal ration (alfalfa pellets). However, Burritt and Provenza (1989) offered LiCl-oats at the same time as the basal ration. Thus, the presence of an alternative food probably influenced the amount of LiCl that sheep voluntarily consumed.

EXPERIMENT 2¹

In experiment 1, sheep experienced a food with a constant flavor and toxicity prior to testing. However, it may be more common for herbivores to gain experience while sampling individual plants or plant parts that vary in both flavor and toxicity. In experiment 2, it was asked, if sheep sample foods with varying intensities of the same

¹ This experiment was conducted by E.A. Burritt to complement the other experiments in this chapter. It is presented here with her permission. I participated in the design of the experiment and am solely responsible for its analysis and summary.

flavor, and then experience gastro-intestinal illness, do they then avoid foods with greater concentrations of the flavor? This question was examined with both a sweet (sodium saccharin) and bitter (aluminum sulfate, i.e., alum) flavor.

Materials and Methods. Forty-eight, 5-months-old lambs were individually penned with continuous access to water and salt. Lambs were divided into 2 groups. Lambs in the saccharin group received 100 g each of barley (*Hordeum vulgare*) grain containing 0.5 and 2% saccharin in separate feed-boxes, while lambs in the alum group were offered 100 g each of barley containing 1 and 2.5% alum in separate feed-boxes. Lambs had access to the foods for 15 min for 1 day. Sixteen of the lambs in each group received a single low dose of LiCl (125 mg/kg body weight; duToit et al., 1991). The remaining 8 lambs in each group served as controls. Lambs that ate less than 90 g of each food were dropped from the trial. This resulted in 8 lambs in the saccharin-no LiCl group, 12 lambs in the saccharin-LiCl group, 7 lambs in the alum-no LiCl group, and 12 lambs in the alum-LiCl group.

The test was conducted the following day by offering each lamb 150 g of each of the foods they ate the previous day. Lambs were allowed to eat for 15 min. or until all of one of the feeds was eaten. A preference index was calculated by dividing the amount of the feed with the low flavor concentration eaten by the total amount of feed eaten (high and low concentration).

A 2-factor ANOVA was used to compare the saccharin with alum flavor and examine the influence of LiCl following consumption on the preference for barley with the low flavor concentration. A t-test was used to test if lambs ate significantly less of

the high than the low concentration within the saccharin and alum groups. This was done by comparing preference for the feed with the low flavor concentration to 0.5 or neutral preference.

Results. Lambs that received LiCl after eating flavored barley showed a higher preference for barley with the low flavor concentration than control lambs on the test day ($P = 0.003$; Figure 3). This result was the same for both saccharin- and alum-flavored barley ($P = 0.214$) and there was no flavor by treatment interaction ($P = 0.696$).

Control lambs that ate saccharin-treated barley exhibited equal preference for both foods on the test day ($P = 0.914$), while lambs in the treatment group preferred barley with the low saccharin concentration ($P = 0.001$). Lambs exposed to alum-treated barley preferred barley with the low alum concentration regardless of whether barley consumption had been followed by LiCl ($P < 0.001$) or not ($P = 0.017$). This indicates that alum had some negative gastro-intestinal consequences that caused a mild CFA.

Discussion. These results show that the flavor-consequence mechanism operating in experiment 1 was effective even when animals initially sampled foods of varying flavor concentration. This could explain the results of Distel and Provenza (1991) who examined intake of old growth and current- season growth of blackbrush (*Coleogyne ramosissima*) by goats. On day 1, goats ate equal amounts of old growth and current-season growth blackbrush. However, on day 2 goats preferred old growth, which has a lower concentration of condensed tannins than current-season growth. It is possible that differences in the amount of condensed tannins creates differences in flavor. Thus,

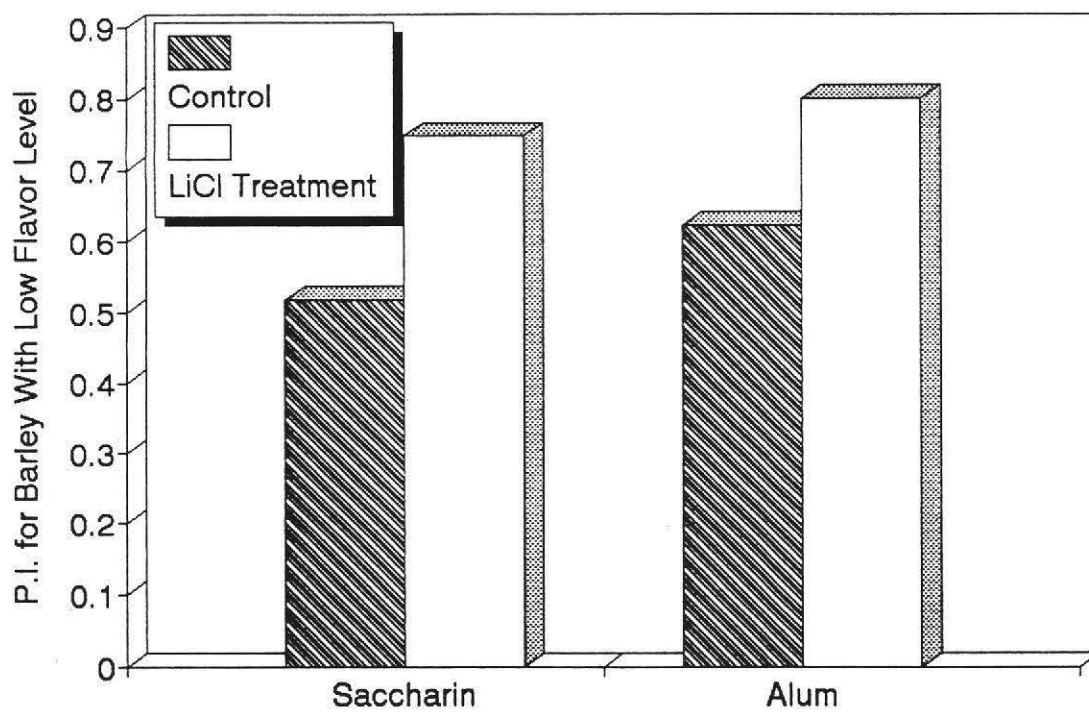


Fig. 3. Preference indices (P.I.) for barley with the low flavor level of saccharin or alum by control and treatment lambs in experiment 2. Preference equals the amount of barley eaten with the low flavor divided by the total amount of flavored barley eaten.

after day 1, when animals experienced illness, they preferred blackbrush with the low concentration of condensed tannins.

Sweet flavors are generally considered feeding attractants while bitter flavors are usually considered feeding deterrents (Garcia and Hankins, 1977). But, the results of experiment 2 suggest that an aversion is as easily formed to a sweet flavor as to a bitter flavor. Thus, sweetness or bitterness may influence the initial acceptance of a food but experience can alter future palatability. Sweet flavors may become aversive and bitter flavors may become highly preferred (Molyneux and Ralphs, 1992).

EXPERIMENT 3

Flavor may not always be a good indicator of toxicity because plants vary in both flavor and toxicity. For instance, minute changes in stereochemistry, which may be difficult for herbivores to detect through taste and smell, can dramatically change the toxicity of many phytochemicals (Bryant et al., 1992; Provenza et al., 1992a). Likewise, some compounds may be present in such minute amounts that they add little or nothing to the overall flavor of the plant (Manners et al., 1992). Finally, plant toxicity may be determined by a group of phytochemicals rather than a single toxin (Bryant et al., 1992). Changes in toxicity involving several phytochemicals may not be well correlated with changes in plant flavor. Additionally, animal characteristics such as diet selection or nutritional state may alter plant toxicity while flavor remains unchanged (Freeland and Janzen, 1974; Brattsten, 1979). Finally, the formation of CFAs is not a selective process in that the flavor preceding illness is not necessarily associated with the cause

of the illness (Garcia, 1989). Thus, an animal may become averted to a flavor that is not associated with any toxic plant component. In this experiment, I asked, how does variability of toxicity influence food ingestion when food flavor remains constant?

Materials and Methods. Thirty 7-months-old lambs were penned individually and offered alfalfa pellets *ad libitum* for 1 hour every morning. They had access to water and salt at all times.

Each lamb was offered 1% of its body weight of ground barley with ground oregano (1.5% concentration) for 30 minutes in the afternoon. Following exposure to oregano-barley, animals received LiCl in gelatin capsules administered with a balling gun. The amount of LiCl depended on treatment as follows: (1) medium control, LiCl administered at 1.5% of amount eaten, (2) high control, LiCl administered at 2.25% of amount eaten, (3) variable treatment, LiCl administered at 0.75, 1.5, or 2.25% of amount eaten on a random schedule. The variable treatment group received LiCl at an average rate equal to 1.5% of the amount eaten. The actual dosing schedule is presented in Table 2.

Table 2. Schedule of LiCl dose (% of amount of barley eaten) received by lambs in the variable treatment during experiment 3.

	Day of Experiment									
	1	2	3	4	5	6	7	8	9	10
Dose	1.50	0.75	2.25	1.50	0.75	1.50	2.25	2.25	0.75	none

The effect of variable LiCl dose on food intake was tested by comparing the intake of the variable treatment group with the high and medium controls during the last 6 days of the trial. The first 4 trial days were not analyzed to allow animals to adjust to the experimental conditions.

Statistical comparison was accomplished with a repeated measures ANOVA with day as the repeated measure. Fisher's LSD on the mean intake of each animal over the 6 trial days was used to separate treatment means.

Results. LiCl treatment strongly influenced intake during the trial ($P < 0.001$; Figure 4). Lambs in the medium control group ate more than lambs in the high control or variable treatment, but intake of the high and variable groups was indistinguishable (Figure 4). All treatments increased intake throughout the trial (i.e. significant day effect; $P < 0.001$) and there was no treatment by day interaction ($P = 0.585$).

Discussion. In this experiment, lambs in the variable treatment were given LiCl at an average rate equal to the medium control. However, they acquired an aversion equivalent to the high control. This response pattern reduced the risk of sheep over-ingesting LiCl.

CONCLUSION AND SUMMARY

Generalist herbivores, such as sheep, apparently have several mechanisms for regulating intake of toxic plants regardless of whether or not toxic variation can be detected through flavor changes. When changes in flavor correspond to changes in toxicity, animals adjusted intake based on an increase or decrease in phytotoxin

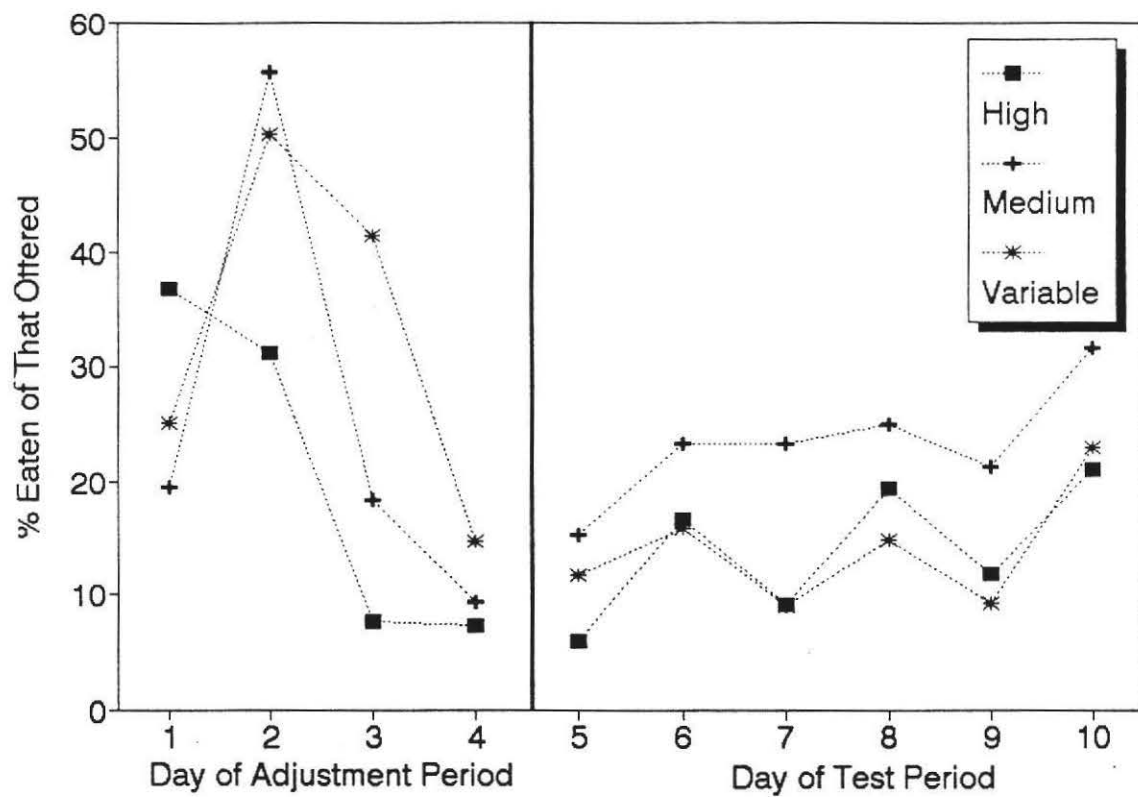


Fig. 4. Percent eaten of oregano-flavored barley offered to lambs in the high, medium, and variable treatments during the adjustment and test periods of experiment 3.

concentration. When toxic variation was not detectable through flavor, animals adopted a conservative strategy of eating an amount based on the maximum toxin dose they had experienced. These results are a step in the direction of understanding how generalist herbivores deal with variability in herbage toxicity.

Based on these results, situations can be predicted in which herbivores are likely to over-ingest plant phytotoxins. First, if animals have not previously encountered a particular phytotoxin, they lack the experience necessary to adjust intake to a level below a lethal dose. Second, animals may over-ingest toxins when conditions are created that make a plant abruptly more toxic but flavor remains unchanged. Finally, the diet selection mechanisms examined in this paper are only operational if the phytotoxin affects the emetic system of the animal (ie. causes gastro-intestinal illness). There are no known mechanisms for developing aversions to plants that are poisonous but do not have negative gastro-intestinal consequences (Provenza et al., 1992a). Thus, plants that do not affect the emetic system can be highly toxic but animals cannot learn to avoid them.

LITERATURE CITED

- Brattsten, L.B. 1979. Biochemical defense mechanisms in herbivores against plant allelochemicals, pp. 200-270, *in* G.A. Rosenthal and D.H. Janzen (eds.). *Herbivores: Their Interaction with Secondary Plant Metabolites*. Academic Press, New York.
- Bryant, J.P., Reichardt, P.B., and Clausen, T.P. 1992. Chemically mediated interactions between woody plants and browsing mammals. *J. Range Manage.* 45:18-24.
- Burritt, E.A., and Provenza, F.D. 1989. Food aversion learning: Ability of lambs to distinguish safe from harmful foods. *J. Anim. Sci.* 67:1732-1739.
- Distel, R.A., and Provenza, F.D. 1991. Experience early in life affects voluntary intake of blackbrush by goats. *J. Chem. Ecol.* 17:431-450.
- duToit, J.T., Provenza, F.D., and Nastis, A. 1991. Conditioned taste aversions: How sick must a ruminant get before it learns about toxicity in foods? *Appl. Anim. Behav. Sci.* 30:35-46.
- Freeland, W.F., and Janzen, D.H. 1974. Strategies in herbivory by mammals: The role of plant secondary compounds. *Am. Nat.* 108:269-289.
- Garcia, J. 1989. Food for Tolman: Cognition and cathexis in concert, pp. 45-85, *in* T. Archer and L. Nilsson (eds.). *Aversions, Avoidance and Anxiety*. Lawrence Erlbaum Assoc., Hillsdale, NJ.
- Garcia, J., and Hankins, W.G. 1977. On the origin of food aversion paradigms, pp. 3-26, *in* L. Barker, M. Domjam and M. Best (eds.). *Learning Mechanisms in Food Selection*. Baylor Univ. Press, Waco, TX.
- Harborne, J.B. 1991. The chemical basis of plant defense, pp. 45-59, *in* R.T. Palo and C.T. Robins (eds.). *Plant Defenses Against Mammalian Herbivory*. CRC Press Inc., Boca Raton, FL.
- Laca, E.A., and Demment, M.W. 1991. Herbivory: The dilemma of foraging in a spatially heterogeneous food environment, pp. 29-44, *in* R.T. Palo and C.T. Robins (eds.). *Plant Defenses Against Mammalian Herbivory*. CRC Press Inc., Boca Raton, FL.

- Laycock, W.A., Young, J.A., and Ueckert, D.N. 1988. Ecological status of poisonous plants on rangelands, pp. 27-42, *in* L.F. James, M.H. Ralphs, and D.B. Nielson (eds.). *The Ecology and Economic Impact of Poisonous Plants on Livestock Production*. Westview Press, Boulder, CO.
- Manners, G.D., Pfister, J.A., Ralphs, M.H., Panter, K.E., and Olsen, J.D. 1992. Larkspur chemistry: toxic alkaloids in tall larkspurs. *J. Range Manage.* 45:63-66.
- McArthur, C., Hagerman, A.E., and Robbins, C.T. 1991. Physiological strategies of mammalian herbivores against plant defenses, pp. 103-114, *in* R.T. Palo and C.T. Robbins (eds.). *Plant Defenses Against Mammalian Herbivory*. CRC Press Inc., Boca Raton, FL.
- McKey, D. 1979. The distribution of secondary compounds within plants, pp. 56-133, *in* G.A. Rosenthal and D.H. Janzen (eds.). *Herbivores: Their Interactions with Secondary Plant Metabolites*. Academic Press, New York.
- Meyer, M.W., and Karasov, W.H. 1991. Chemical aspects of herbivory in arid and semiarid habitats, pp. 167-187, *in* R.T. Palo and C.T. Robbins (eds.). *Plant Defenses Against Mammalian Herbivory*. CRC Press Inc., Boca Raton, FL.
- Molyneux, R.J., and Ralphs, M.H. 1992. Plant toxins and palatability to herbivores. *J. Range Manage.* 45:13-18.
- Nowlis, G.H. 1974. Conditioned stimulus intensity and acquired alimentary aversion in the rat. *J. Comp. and Physiol. Psychol.* 86:1173-1184.
- Provenza, F.D., and Balph, D.F. 1990. Applicability of five diet-selection models to various foraging challenges ruminants encounter, pp. 423-460, *in* R.N. Hughes (ed.). *Behavioral mechanisms of food selection*. Springer-Verlag, Berlin.
- Provenza, F.D., Balph, D.F., Dwyer, D.D., Ralphs, M.H., and Pfister, J.A. 1988. Toward understanding the behavioral responses of livestock to poisonous plants, pp. 407-424, *in* L.F. James, M.H. Ralphs, and D.B. Nielsen (eds.). *The Ecology and Economic Impact of Poisonous Plants on Livestock Production*. Westview Press, Boulder, CO.
- Provenza, F.D., Pfister, J.A., and Cheney, C.D. 1992a. Mechanisms of learning in diet selection with reference to phytotoxicosis in herbivores. *J. Range Manage.* 45:36-45.
- Provenza, F.D., Lynch, J.J., and Nolan, J.V. 1992b. Food aversion conditioned in anesthetized sheep. *J. Comp. Physiol. Psych.* (submitted).

- Robbins, C.T., Mole, S., Hagerman, A.E., and Hanley, T.A. 1987. Role of tannins in defending plants against ruminants: Reduction in dry matter digestion? *Ecology* 68:1606-1615.
- Scott, T.R., and Giza, B.K. 1987. A measure of taste intensity discrimination in the rat through conditioned taste aversions. *Physiol. and Behavior* 41:315-320.
- Spector, A.C., and Grill, H.J. 1988. Differences in the taste quality of maltose and sucrose in rats: Issues involving the generalization of conditioned taste aversions. *Chem. Senses* 13:95-113.

CHAPTER III

CAN PLANTS PRACTICE MIMICRY TO AVOID
GRAZING BY MAMMALIAN HERBIVORES?**Abstract**

Mimicry has been suggested as a grazing avoidance mechanism for plants. This study examined the ability of a mammalian herbivore to generalize conditioned flavor aversions (CFAs) to determine if the conditions for plant mimicry exist. Nine sheep (treatment group) were averted to cinnamon on ground rice while an additional 9 sheep (control group) received cinnamon on rice with no negative post-ingestive consequences. When offered a choice between wheat and cinnamon-flavored wheat the control group ingested more ($P < 0.05$) cinnamon-flavored wheat ($45 \pm 6\%$) than did the treatment group ($3 \pm 1\%$) in four test periods. This implies that herbivores generalize CFAs and thus non-poisonous plants could mimic the flavor of poisonous plants to avoid grazing. Next, the animals were given a choice between soybean meal (SBM) in a food box which smelled of cinnamon and SBM in a food box with no added odor. The treatment group ate less ($P < 0.05$) SBM with cinnamon odor than did the control group in the first test period ($13 \pm 10\%$ vs. $58 \pm 11\%$). However, the following three periods revealed no intake differences between control and treatment animals. This suggests that odor alone is not persistently effective in preventing herbivory by sheep, but that both taste and odor must be similar for one plant to successfully mimic another.

Introduction

Many animals contain toxins that protect them from predation. Some non-toxic animals mimic the color or appearance of toxic species to repel predators. Perhaps the best known example of mimicry is that of the viceroy butterfly (*Limenitis archippus archippus*) which mimics the appearance of the toxic monarch butterfly (*Danaus plexippus*; Brower 1969).

Flavor mimicry to avoid predation has been suggested for plants, but has not yet been documented (Wiens 1978, Rhoades 1979, Eisner and Grant 1981). However, other forms of plant mimicry are known. Some plants smell like rotting flesh, which attracts insect pollinators. Several orchid species (e.g., *Ophrys spp.*) "look" like female bees to attract pollinators. Still other plants closely resemble stones to avoid being eaten (Wiens 1978, Barrett 1987).

Most mammalian herbivores learn to avoid harmful foods based on odor and taste rather than appearance (Garcia 1989). Therefore, one would expect the evolution of non-toxic plant species that imitate the odor and (or) taste of harmful species (Batesian mimicry). Likewise, the convergence of flavor by groups of harmful species (Muellerian mimicry) has also been hypothesized (Brower 1969, Rhoades 1979).

Successful mimicry requires: (1) that predators can associate negative consequence of ingesting the mimetic model with some visual, olfactory, or taste cue, (2) that predators can generalize this cue-consequence association from the model to a mimic, and (3) that the mimic and model are present in the predator's feeding area to reinforce learning via occasional ingestion of the model. It is well-established that

mammalian herbivores can associate post-ingestive consequences with plant characteristics - the first requirement for mimicry (Garcia 1989). The objective of this study was to examine the second requirement - that a mammalian herbivore can generalize the effect of a toxin from one food to another based on flavor. I first examined flavor (taste with odor) as a mimetic cue, then odor alone as a sensory mode for mimicry.

Flavor mimicry experiment

The senses of taste and smell are vitally important in diet selection by mammalian herbivores (Arnold 1966). Efficient neurological pathways connect gastro-intestinal consequences to the taste and odor of foods (Palmerino et al. 1980, Garcia 1989). Therefore, I began by studying flavor generalization. I asked the question: If sheep develop an aversion to one food will they generalize the aversion to another food with a similar flavor?

Research methods

The study was conducted in three consecutive phases: preconditioning, conditioning, and testing. The preconditioning phase was from days 1 to 3 of the experiment during which 9 sheep (treatment group) were offered 500 g of milo (*Sorghum vulgare* grain) twice a day for 7 min and 9 sheep (control group) were offered ground rice (*Oryza sativa*) with 1% cinnamon (500 g). This preconditioning was necessary to insure that lambs in the control were exposed to rice with cinnamon.

During the conditioning phase, days 4 to 6, the treatment sheep were offered rice with cinnamon (500 g) and the control sheep were offered milo (500 g). Any treatment sheep that ate more than 10 g was given about 250 mg/kg body weight of lithium chloride in a gelatin capsule administered with a balling gun. Lithium chloride at this dose consistently causes food aversions in sheep (duToit et al. 1991). The control sheep were also given lithium chloride, if more than 10 g of milo was eaten, to control for changes in food ingestion caused by lithium chloride.

In the testing phase, days 7 and 8, each sheep was offered a choice between ground wheat plus cinnamon and plain ground wheat (*Triticum aestivum*) twice a day for 7 min, resulting in 4 test periods. The test variable was the amount of wheat with cinnamon eaten expressed as a percent of the total wheat (plain and with cinnamon) eaten.

I compared the treatment and control groups statistically with a repeated measures analysis of variance, with test period as the repeated measure. The test variable was analyzed after arcsine transformation, to meet normality assumptions. Sheep were the experimental subjects in this research because they are readily available, easily handled, and information is available on the establishment of conditioned flavor aversions in sheep (see, Provenza and Balph 1990).

Results

Treatment sheep ate a lower proportion of wheat with cinnamon than control sheep in all test periods ($P < 0.01$; Fig. 5). Neither the test period ($P = 0.48$) nor the treatment

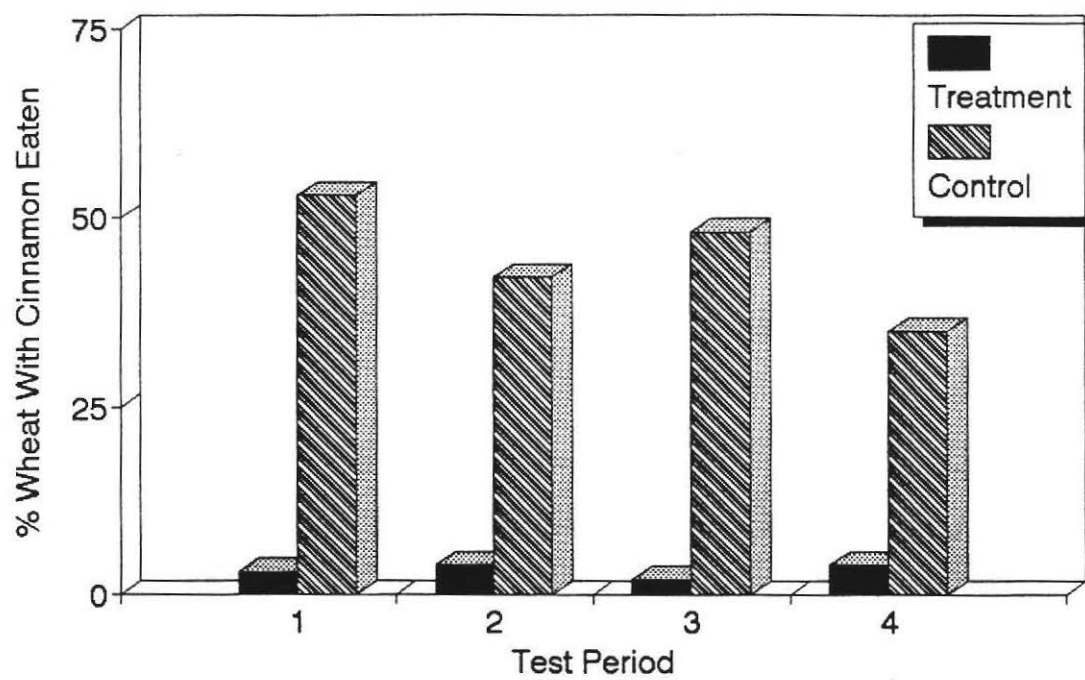


Figure 5. Percent of cinnamon-flavored wheat eaten by sheep simultaneously offered plain wheat and cinnamon-flavored wheat (least significant difference for repeated measures design = 22.8).

by period interaction ($P = 0.29$) were significant. Subsequent experiments established that sheep are able to discriminate between wheat and rice, and wheat with cinnamon and rice with cinnamon. Thus, these data support the hypothesis that herbivores generalize conditioned flavor aversions from one food to another based on flavor.

Odor mimicry experiment

In theory, a plant would lose less foliage when grazed if it mimicked the odor, rather than taste, of a poisonous plant because tissue removal is not required to detect odor. Eisner and Grant (1981) hypothesized that since many plant toxins are nonvolatile, and hence odorless, salient odors may have evolved in some poisonous plants to advertise toxicity. Additionally, mimicry of these advertising odors may have evolved in some plants.

In a second experiment, I studied the role of odor in the generalization of conditioned flavor aversions. I asked: Will sheep that have been averted to cinnamon on one food generalize the aversion to a new food based on the odor alone?

Research methods

This experiment was conducted with the same sheep used in the flavor experiment. In the conditioning phase, the conditioning procedure as employed in the flavor experiment was repeated for 2 days. The testing phase consisted of offering all sheep a choice between plain soybean meal (ground *Glycine max*) and soybean meal with the odor of cinnamon.

Cinnamon odor was presented by saturating a slice of white bread with cinnamon and placing it behind a screen in a food container. A slice of white bread without cinnamon was placed behind a screen in the plain soybean meal food container to control for the odor of bread. The tests were conducted in the morning and evening of 2 days resulting in 4 test periods.

Results

Treatment sheep ate a lower proportion of soybean meal with cinnamon odor than control sheep during the first test period ($P < 0.01$; Fig. 6). But, there were no differences in the proportion eaten during test period 2, 3, and 4 (Fig. 6). The treatment by test interaction was significant ($P = 0.02$). These data indicate that mimicry of odor alone apparently would not be effective as a grazing avoidance mechanism, except perhaps the first time the plant is encountered by an herbivore that has been averted.

Discussion

Flavor mimicry has been suggested by several authors as a plausible means of grazing avoidance by plants (Atsatt and O'Dowd 1976, Wiens 1978, Rhoades 1979). However, for flavor mimicry to be effective, herbivores must generalize the effect of a toxin from one plant to another based on flavor. This investigation of flavor generalization by a mammalian herbivore indicates that mimicry may be an effective anti-herbivore mechanism.

Eisner and Grant (1981) argued convincingly that olfactory mimicry in plants is a logical analog of visual mimetic systems in animals, and may, in fact, be

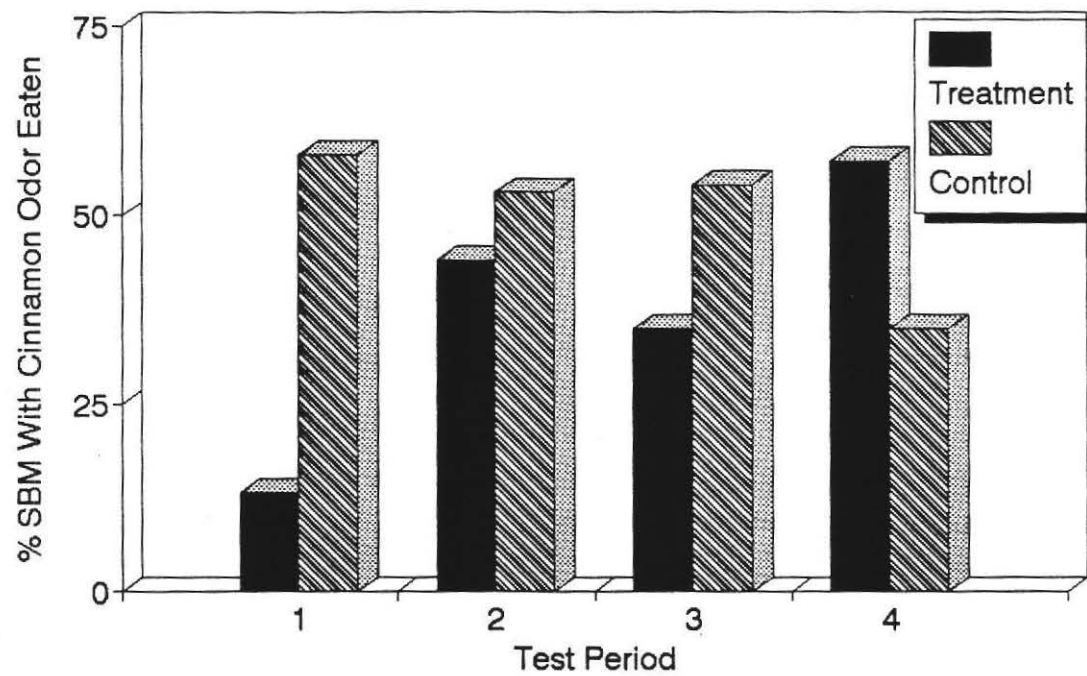


Figure 6. Percent soybean meal with the odor of cinnamon eaten by sheep simultaneously offered soybean meal with and without the odor of cinnamon (least significant difference for repeated measures design = 31.1).

"commonplace." However, closer examination of odor generalization by a mammalian herbivore in my study suggested that odor mimicry is not likely to be effective because of the relationship between odor and taste in flavor aversions. Understanding how flavor and (or) odor mimicry could work as an anti-herbivore mechanism requires knowledge of the roles flavor and odor play in food selection.

It is well established that when illness results from consuming a novel food, both the taste and odor of the food become aversive, and both are subsequently avoided (reviewed by Garcia 1989). When odor alone is paired with illness, the odor does not become aversive (Palmerino et al. 1980). Thus, odor is only important in food selection when accompanied by taste; odor is "potentiated" by taste (Rusiniak et al. 1979).

For odor mimicry to be effective, herbivores would need to avoid plants with similar odors but different tastes. The depressed intake by the treatment group on the first day of the odor trial was consistent with results of Palmerino et al. (1980), and suggests that odor mimicry would be an effective anti-herbivore mechanism. However, Palmerino et al. (1980) conducted only one test period; I continued testing for 3 additional periods. These additional results suggested that olfactory mimicry would not necessarily be an important mechanism for plants to avoid grazing by mammalian herbivores.

After the initial test period, the aversion to cinnamon odor was quickly extinguished. Two factors may have been involved in this rapid extinction. First, the sheep in the treatment group may have tasted enough soybean meal to override the odor of cinnamon in the food box. Secondly, all of the treatment sheep ate more than 70 g of the soybean meal without cinnamon odor in the first test period. Thus, by the

second test period, soybean meal may have become a safe, familiar food and its cinnamon odor was disregarded.

If flavor mimicry is a plausible anti-herbivore mechanism, why then, are there no examples? It is possible that flavor mimicry occurs but has simply not been discovered. We cannot effectively analyze how herbivores perceive the flavors of different plants. Therefore, scientists have not considered assessing the response of mammalian herbivores to plants with similar flavors but different post-ingestive consequences.

On the other hand, mimicry may not be sufficiently deceptive to "fool" mammalian herbivores. Sheep (Thorhallsdottir et al. 1987, duToit et al. 1991), goats (Provenza et al. 1990, Distel and Provenza 1991), and cattle (Pfister et al. 1991) do not refuse to eat otherwise nutritious plants that contain toxins; instead, they regulate intake of these plants in accordance with their toxicity. Such studies also show that herbivores sample foods and determine the amount they can safely ingest.

If flavor mimicry exists in the plant kingdom, it may be revealed in palatability anomalies. For example: mimicry may result in nutritious plants that have unexpectedly low palatabilities; or non-toxic plants that are avoided by mammalian herbivores in one area and preferred in another if the poisonous model plant occurs in the first area but not the latter.

Literature cited

- Arnold, G.W. 1966. The special senses in grazing animals. I. Sight and dietary habits in sheep. - *Aust. J. Agric. Res.* 17:521-529.
- Atsatt, P.R. and O'Dowd, D.J. 1976. Plant defense guilds. - *Science* 193:24-29.
- Barrett, S.C. 1987. Mimicry in plants. - *Sci. American* 257:76-83.
- Brower, L.P. 1969. Ecological chemistry. - *Sci. American* 220:22-29.
- duToit, J.T., Provenza, F.D. and Nastis, A. 1991. Conditioned taste aversions: how sick must a ruminant get before it detects toxicity in foods? - *Appl. Anim. Behav. Sci.* 30:35-46.
- Distel, R.A. and Provenza, F.D. 1991. Experience early in life affects voluntary intake of blackbrush by goats. - *J. Chem. Ecol.* 17:431-450.
- Eisner, T. and Grant, R.P. 1981. Toxicity, odor aversions, and "olfactory aposematism". - *Science* 312:476.
- Garcia, J. 1989. Food for Tolman: cognition and cathexis in concert. - In: Archer, T. and Nilsson, L. (eds), *Aversion, avoidance and anxiety*. Lawrence Erlbaum Assoc., Hillsdale, N.J., pp. 45-85.
- Palmerino, C.C., Rusiniak, K.W. and Garcia, J. 1980. Flavor-illness aversions: the peculiar roles of odor and taste in memory for poison. - *Science* 208:753-755.
- Pfister, J.A., Provenza F.D. and Manners, G.D. 1991. Ingestion of tall larkspur by cattle: separating effects of flavor from postingestive consequences. - *J. Chem. Ecol.* 16:1697-1705.
- Provenza, F.D. and Balph, D.F. 1990. Applicability of five diet-selection models to various foraging challenges ruminants encounter. - In: Hughes, R.N. (ed), *Behavioral mechanisms of food selection*. NATO ASI Series, Vol G20. Springer-Verlag, Berlin, pp. 423-259.
- Provenza, F.D., Burritt, E.A., Clausen, T.P., Bryant, J.P., Reichardt, P.B. and Distel, R.A. 1990. Conditioned flavor aversion: a mechanism for goats to avoid condensed tannins in blackbrush. - *Am. Nat.* 136:810-828.

- Rhoades, D.F. 1979. Evolution of plant chemical defense against herbivores. In: Rosenthal, G.A. and Janzen, D.H. (eds), *Herbivores: their interaction with secondary plant metabolites*. Academic Press, New York, NY, pp. 3-53.
- Rusiniak, K.W., Hankins, W.G., Garcia, J. and Brett, L.P. 1979. Flavor-illness aversions: potentiation of odor by taste in rats. - *Behav. and Neur. Biol.* 25:1-17.
- Thorhallsdottir, A.G., Provenza, F.D. and Balph, D.F. 1987. Food aversion learning in lambs with or without a mother: discrimination, novelty and persistence. - *Appl. Anim. Behav. Sci.* 18:327-340.
- Wiens, D. 1978. Mimicry in plants. - *Evol. Biol.* 11:365-403.

CHAPTER IV

THE EFFECT OF FLAVOR INTENSITY AND TOXIN DOSE ON
THE FORMATION AND GENERALIZATION
OF FLAVOR AVERSIONS IN SHEEP

Abstract

When an animal experiences gastro-intestinal illness after eating a novel food, it develops a dislike for the food called a conditioned flavor aversion (CFA). Understanding flavor aversions is important for diet training management, understanding animal response to poisonous plants, and helping animals adjust to new foraging environments. The objectives of this research were to determine how variation in (1) flavor concentration, and (2) dose of a gastro-intestinal toxin affect the establishment of CFAs in sheep. This research also examined how the formation of a CFA to one food influences the consumption of another food with a similar flavor and an apparently novel food. Although the role of flavor aversions in the acceptance of harvested or processed rations has seldom been studied, I will present data that suggests this may be a costly oversight. In a series of experiments with lambs using grains and the added flavor - oregano, I determined that: (1) lambs generalize aversions from familiar to novel foods when both foods have a flavor in common; (2) the more ill an animal gets after eating a food, the stronger is the CFA formed to the

food, the greater the generalization of that aversion to a similar food, and the greater the avoidance of a novel food; (3) the intensity of food flavor apparently did not affect the acquisition of generalization of a CFA in this experiment. However, food flavor did influence the acceptance of a novel food.

Introduction

The study of diet selection has in large part been fueled by the need for information to manage animal nutrition. In the case of domestic livestock, the first diet studies were initiated to answer the question, "What do animals eat?" (Heady and Torell, 1959; Arnold, 1962; Grant et al., 1976). These studies revealed that animals consistently selected diets higher in nitrogen, digestibility, and metabolizable energy, and lower in crude fiber and phytotoxins than herbage available for consumption (reviewed by Arnold and Dudzinski, 1978).

More recently, research has focused on the mechanism of diet selection to determine the basis on which animals make dietary decisions. One area of interest pertains to how animals learn to avoid eating toxic foods. We now know that negative post-ingestive consequences can alter food preferences in ruminants via conditioned flavor aversions (CFAs; Burritt and Provenza, 1989a; Lane et al., 1990; Provenza et al., 1992). Understanding food aversions is important for diet training management (Provenza and Balph, 1987), for mediating animal damage (Gustavson et al., 1982; Nicolaus and Nellis, 1987), and for understanding animal response to poisonous plants (Provenza et al., 1988; 1992).

The strength and persistence of flavor aversions depend on gastro-intestinal parameters such as severity of illness (duToit et al., 1991) and the delay between ingestion and illness (Burritt and Provenza, 1992). Comparatively little is known about the flavor attributes that influence the acquisition of CFAs. The effect of flavor intensity on the formation of a CFA has seldom been researched (Dragoin, 1971).

One area of feeding behavior that has received little research attention is how the consequences of eating one food influence the consumption of other foods. Results from experiments in chapter three demonstrate that sheep generalized aversions from one food to another based on a common flavor. Animals also express heightened neophobia after experiencing gastro-intestinal distress from eating a new food (duToit et al., 1991). Thus, novelty can also be considered a basis for generalization (Schlenoff, 1984). The properties of flavor or gastro-intestinal feedback that affect the generalization of CFA's or the acceptance of novel foods are largely unknown.

The objectives of this research were to determine how variation in: (1) flavor concentration, and (2) dose of a gastro-intestinal toxin affect the establishment and generalization of CFA's in sheep.

Materials and Methods

I used 78 lambs that were crosses of Columbia, Rambouillet, Suffolk, Targhee, and Finnish Landrace breeds. Lambs were obtained as 3- to 7-day-old orphans from the U.S. Sheep Experiment Station at Dubois, ID. Lambs were fed a commercial milk-replacer diet until 2.5 weeks-of-age then were weaned to a mix of ground alfalfa,

soybean meal, milo, and molasses. These were the only feeds that lambs were exposed to prior to the experiment. The research was initiated when lambs were about 4 months old with an average weight of 31 kg (SE=0.6).

Lambs were randomly assigned to 1 of 3 experiments (26 lambs/experiment) that were conducted simultaneously. The experiments were designed to examine the influence of flavor intensity and dose of gastro-intestinal toxin on: (1) the acquisition of a CFA, (2) the generalization of a CFA to another food with a similar flavor, and (3) the acceptance of a novel food. The average weights of lambs in each experimental group were similar. Each experiment was a 2 × 2 factorial with 2 levels of flavor and 2 levels of toxin dose.

Adjustment Period. Lambs were individually penned 12 days before the experiment in order for them to adjust to the pens and feeding schedule. Pens were about 3 m² in area set side-by-side to allow social contact among lambs. Alfalfa pellets were offered to each lamb *ad libitum* for one hour in the morning (0700 h) and in the evening (1700 h) of each day. At 1100 h each day, lambs were offered 300 g of a novel food for 5 minutes to prepare them to accept a novel food during the experiment. Subjects were offered each of 4 novel foods (Calf Manna², cracked corn, whole rice, and wheat bran) for 3 consecutive days (a total of 12 days).

Experiment 1 - Formation of a CFA. On the first day following the adjustment period, all lambs (n=26) were offered alfalfa pellets in the morning and evening as they

² Calf manna is a concentrate-based pelleted feed produced by the Manna Pro Company.

had been during the adjustment period. At 1100 h, half the lambs were offered 250 g of ground barley with 1% oregano powder by weight and the other half received barley with 3% oregano. Oregano was selected because preliminary experiments indicated that, at these concentrations, it was not aversive to lambs. The concentrations of oregano were based on reports from co-workers who tasted the barley/oregano mix and described 1 and 3% as a "slight" and "strong" oregano flavor.

Thirty minutes after exposure to oregano-barley, lambs were given either 100 or 225 mg/kg body weight of lithium chloride (LiCl). LiCl, at these dosages, is a nonlethal gastro-intestinal toxin that produces mild or strong CFAs in lambs, respectively (duToit et al., 1991). Two lambs were removed from this experiment because they ate less than 10 g of oregano-flavored barley. This resulted in 6 lambs that ate barley with 1% oregano and received the high dose of LiCl, 6 lambs that ate barley with 1% oregano and received the low dose of LiCl, 6 lambs that ate barley with 3% oregano and received the high dose of LiCl, and 6 lambs that ate barley with 3% oregano and received the low dose of LiCl.

The test was conducted at 1100 h on the second day after the LiCl conditioning. Each lamb was offered 250 g of ground barley with the same concentration of oregano it had previously consumed. This allowed me to examine the strength of the CFA to oregano-flavored barley. The test was repeated on the following day to see if treatment effects persisted for a second day.

Experiment 2 - Generalization of a CFA. The LiCl conditioning procedure was the same for this experiment as for the previous experiment. Again, 2 lambs were removed

from the experiment because they did not consume a sufficient amount of oregano-flavored barley. The test was conducted on the second and third days after conditioning when all lambs (n=24) were offered oregano-flavored wheat rather than oregano-flavored barley, as in experiment 1. This enabled me to assess the degree to which lambs generalized a CFA from barley to a new food, wheat, when both contained oregano.

Experiment 3 - Acceptance of a Novel Food. In this experiment 26 lambs were offered 250 g of ground barley without oregano for 5 min on day 1. Two lambs were randomly removed from the experiment to keep the sample size equal to experiments 1 and 2. Following ingestion of barley, 12 lambs received 100 mg/kg and 12 received 225 mg/kg body weight of LiCl. On the second day after the LiCl conditioning, 6 lambs that had received the low LiCl dose were offered 250 g of wheat with 1% oregano and the other 6 lambs were offered barley with 3% oregano. Similarly, the lambs that received the high dose of LiCl were offered wheat with either 1% (6 lambs) or 3% (6 lambs) oregano. This testing procedure was repeated on the following day to see if treatment effects persisted.

Statistical Analysis. The effects of flavor intensity, LiCl dose, and their interaction on the intake of the test food (the food offered on the test days) were examined for each experiment with a factorial analysis of variance (ANOVA). Data from all experiments were combined into an overall ANOVA to compare intake in the CFA, generalization, and novel acceptance experiments. The 2- and 3-way interactions between experiment, flavor level and toxin dose were included in an overall ANOVA.

Results and Discussion

Toxin Dose. The dose of LiCl influenced (1) the formation of a CFA ($P = 0.003$; Exp. 1, Figure 7), (2) the generalization of that aversion ($P = 0.004$; Exp. 2, Figure 7), and (3) the acceptance of a novel food ($P < 0.001$; Exp. 3, Figure 7) on the first test day. The LiCl dose and flavor concentration did not interact in any experiments.

Lambs in all experiments ate more of the test food on the second day than on the first test day. Since treatment effects did not change from the first to the second test day, and since the primary goal of the experiment was to examine the initial acceptance of the test foods, the results of the second test day are not presented here; they are presented in appendix figures 1 and 2.

In this study LiCl dose affected the formation of a CFA to oregano-barley and the consumption of a new food (wheat) that had a similar flavor element (oregano). This can be interpreted as a generalized aversion based on the common flavor (oregano) since overall intake of lambs in the CFA experiment did not differ from lambs in the CFA generalization experiment ($P < 0.05$). Lambs in experiment 3 (novel acceptance experiment) ate more than lambs in experiments 1 or 2. Thus, similarity between the conditioning and test food is more important in determining intake of the test food than the novelty of the test food.

Influence of Flavor Intensity. The concentration of oregano in the test food was not sufficiently different to influence intake in the CFA ($P = 0.690$; Exp. 1, Figure 8) or generalization ($P = 0.468$; Exp. 2, Figure 8) experiments. However, flavor concentration

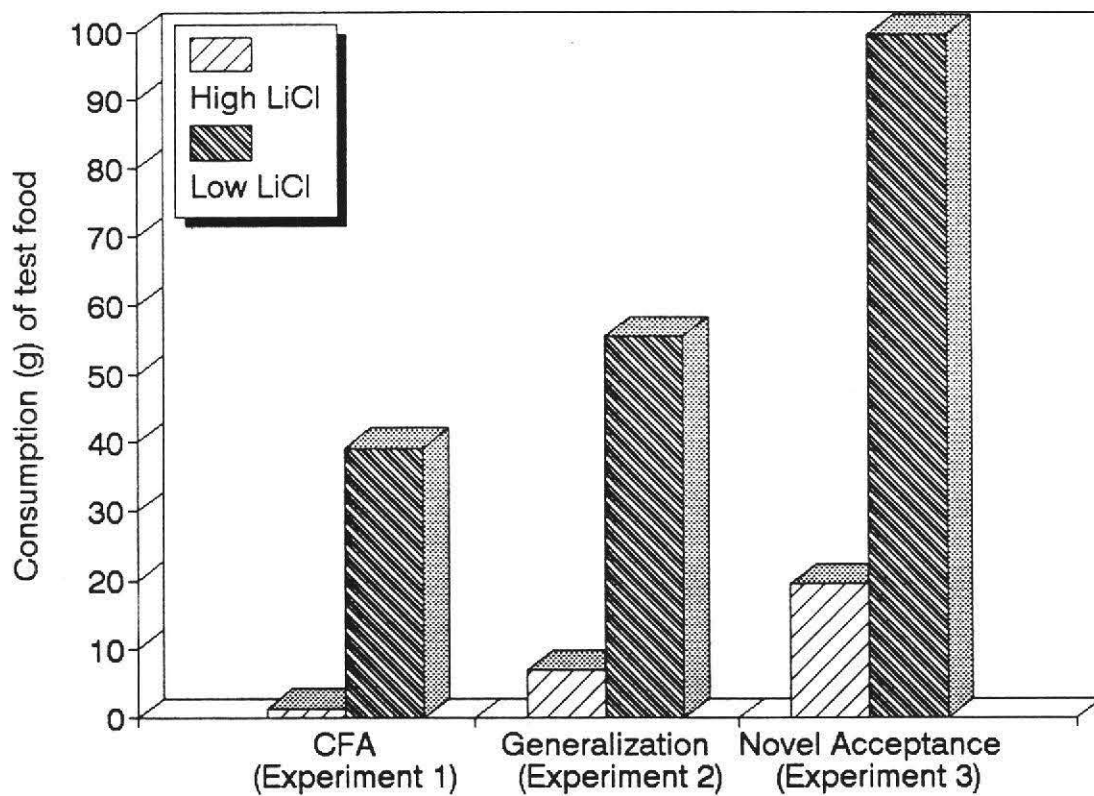


Figure 7. The influence of LiCl dose on the intake of the test foods on the first test day in experiments 1, 2, and 3. Each bar represents mean consumption of animals receiving the same toxin dose averaged over flavor level. A high LiCl dose was 225 mg/kg and a low dose was 100 mg/kg of LiCl.

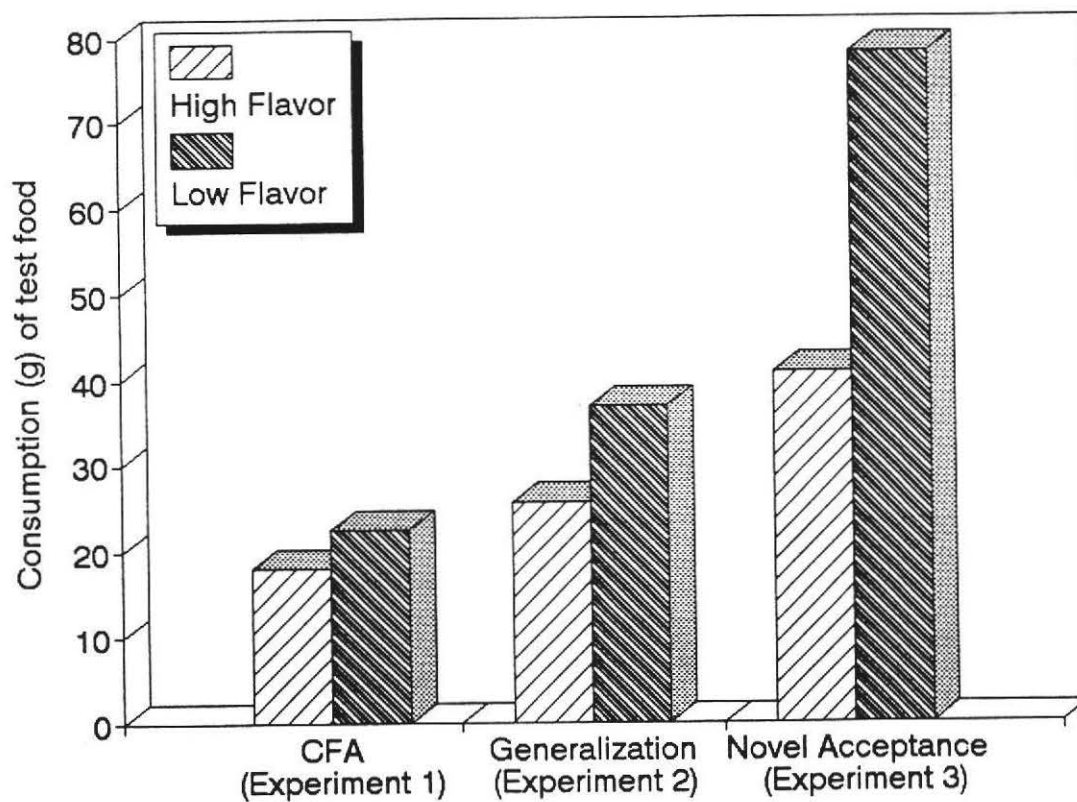


Figure 8. The influence of flavor level on the intake of the test foods on the first test day in experiments 1, 2, and 3. Each bar represents mean consumption of animals receiving the same flavor level averaged over toxin dose. A high level of flavor was 3% ground oregano and a low flavor level was 1% ground oregano.

did influence the acceptance of a novel food ($P = 0.008$; Exp. 3, Figure 8). Less of the wheat with 3% oregano was eaten than wheat with 1% oregano.

Research with rats indicates that CFAs are difficult to establish to weak or non-salient flavors such as vanilla in water (Kalat and Rozin 1970), but that stronger flavors, such as strong salt solutions, result in stronger aversions (Dragoin, 1971; Nowlis 1974). However, my results suggest that flavors need not be intense in order to become good agents for establishing CFAs or for the generalization of CFAs. Nevertheless, there may be cases in nature where flavor qualities influence the strength or generalization of a CFA. It has been suggested that poisonous plants should evolve strong tastes and odors to advertise toxicity (Rhoades, 1979; Eisner and Grant, 1981). This may well be the case but evidence is lacking.

Flavor intensity appears to play an important role in the acceptance of novel foods. This suggests that plants with strong flavors may gain an anti-herbivore advantage since animals may find them initially unacceptable. However, once sampled, flavor intensity may not influence the conditioned preferences or aversions formed to the food (i.e., results of experiment 1).

Management Implications

Livestock managers frequently move animals from familiar to novel foraging environments. Bulls, rams, or replacement females may be transported thousands of miles as part of breeding programs. Lambs and calves also encounter novel foraging environments at weaning time, when they are placed in feedlots for fattening. The

acceptance of safe novel foods and avoidance of toxic foods in these situations can influence weight gain and even survival (Provenza et al., 1988; Tolley et al., 1988; Ortega-Reyes et al., 1992). The results of this research have several implications to the management of livestock in novel environments.

First, animals may generalize flavor/consequence relationships from familiar to novel environments. A new food may look, smell, or taste like a familiar food that is preferred or avoided. In this way, generalized aversions may be a mechanism by which herbivores can avoid novel toxic foods as suggested in chapter 3. Likewise, generalized preferences may increase the intake of safe novel foods (Biscornet et al., 1992). The successful transition of animals to new pastures therefore depends on the proportion of forage plants that are familiar to the animal; or possibly the presence of plants that are similar (i.e., of the same genera) to familiar plants.

Second, this research emphasizes the importance of avoiding incidences that cause gastro-intestinal illness. Since aversions can be formed to any food eaten prior to the onset of illness, even if the food did not cause the illness, it is important to know when management practices inadvertently cause illness. Also, since illness is more readily associated with novel than familiar foods (Burritt and Provenza, 1989b), strong aversions may be formed to novel foods if illness is not carefully managed. For example, when animals are moved to new foraging areas or feedlots they may become ill due to transport conditions or diseases such as shipping fever (pneumatic pasteurellosis; Stamm, 1975). Livestock managers also commonly vaccinate or treat animals for parasites when moving them to new pastures or feedlots. The gastro-

intestinal consequences of these practices are seldom considered. My research shows that how ill an animal gets influences the acceptance of a food, eaten prior to illness, the acceptance of a similar food, and even the acceptance of an apparently novel food.

Finally, the flavor of novel foods may play a critical role in their acceptance. When range animals are placed in feedlots they are usually offered a ration of novel foods. Delayed acceptance of this ration may increase the amount of time animals spend in feedlot before reaching slaughter weight (Ortega-Reyes et al., 1992). Feed additives, such as vitamins, minerals, or antibiotics are often incorporated in feedlot rations. These additives may have strong flavors and if added in sufficient amounts may actually decrease the initial acceptance of the ration. Feedlot rations are usually formulated to meet animal nutrient requirements with a least-cost analysis. Little attention is paid to how the flavor of the resulting ration may influence its acceptance.

In summary, this research indicates that flavor characteristics and gastro-intestinal events following consumption can influence the intake of novel foods. But, much more must be known about the role of flavor in the formation of CFAs in order to guide management decisions. For example, are animals predisposed to forming stronger aversions against bitter flavors than sweet or salty flavors? How strong must a flavor be before it influences the formation of food aversions? If answers to these questions were known, feed rations could be designed to minimize the probability that animals will become averted to them. We must also begin to consider and research the gastro-intestinal effects of many livestock management practices such as the effects of specific vaccines or parasite control agents.

Literature Cited

- Arnold, G.W. 1962. Effects of pasture maturity on the diet of sheep. *Aust. J. Agric. Res.* 13:701.
- Arnold, G.W. and M.L. Dudzinski. 1978. *Ethology of Free-Ranging Domestic Animals.* Elsevier Sci. Pub. Comp., Amsterdam.
- Biscornet, M.J., K.L. Launchbaugh and F.D. Provenza. 1992. Food neophobia: management implications and solutions. In: *Proceedings, Soc. for Range Management 45th Ann. Meeting. Abstr. No. UG013.*
- Burritt, E.A. and F.D. Provenza. 1989a. Food aversion learning: conditioning lambs to avoid a palatable shrub (*Cercocarpus montanus*). *J. Anim. Sci.* 67:650.
- Burritt, E.A. and F.D. Provenza. 1989b. Food aversion learning: ability of lambs to distinguish safe from harmful foods. *J. Anim. Sci.* 67:1732.
- Burritt, E.A. and F.D. Provenza. 1992. Ability of lambs to learn with a delay between food ingestion and consequences given meals containing novel and familiar foods. *Appl. Anim. Behav. Sci.* (In press).
- Dragoin, W.B. 1971. Conditioning and extinction of taste aversions with variation in the intensity of the CS and UCS in two strains of rats. *Psychon. Sci.* 22:303.
- duToit, J.T., F.D. Provenza and A. Nasis. 1991. Conditioned taste aversions: how sick must a ruminant get before it learns about toxicity in foods? *App. Anim. Behav. Sci.* 30:35.
- Eisner, T. and R.P. Grant. 1981. Toxicity, odor aversions, and "olfactory aposematism". *Science* 312:476.
- Gustavson, C.R., J.R. Jowsey and D.N. Milligan. 1982. A 3-year evaluation of taste aversion coyote control in Saskatchewan. *J. Range Manage.* 35:57.
- Grant, S.A., G.R. Bolton and A.J.F. Russel. 1984. The utilization of sown and indigenous plant species by sheep and goats in grazing hill pastures. *Grass and Forage Sci.* 39:361.
- Heady, H.F. and D.T. Torell. 1959. Forage preference exhibited by sheep with esophageal fistulas. *J. Range Manage.* 12:28.

- Kalat, J.W. and P. Rozin. 1970. "Salience": a factor which can override temporal contiguity in taste aversion learning. *J. Comp. and Physiol. Psychol.* 71:192.
- Lane, M.A., M.H. Ralphs, J.D. Olsen, F.D. Provenza and J.A. Pfister. 1990. Conditioned taste aversion: potential for reducing cattle losses to larkspur. *J. Range Manage.* 43:127.
- Nicolaus, L.K. and D.W. Nellis. 1987. The first evaluation of the use of conditioned food aversion to control predation by mongooses upon eggs. *Appl. Anim. Behav. Sci.* 17:329-346.
- Nowlis, G.H. 1974. Conditioned stimulus intensity and acquired alimentary aversion in the rat. *J. Comp. and Physiol. Psychol.* 86:1173.
- Ortega-Reyes, L., F.D. Provenza, C.F. Parker and D.F. Balph. 1992. Drylot performance and ruminal papillae development of lambs exposed to a high concentration diet while nursing. *Small Rum. Res.* (In press).
- Provenza, F.D. and D.F. Balph. 1987. Diet learning by domestic ruminants: theory, evidence and practical implications. *Appl. Anim. Behav. Sci.* 18:211.
- Provenza, F.D., D.F. Balph, J.D. Olsen, D.D. Dwyer, M.H. Ralphs and J.A. Pfister. 1988. Toward understanding the behavioral responses of livestock to poisonous plants. In: L.F. James, M.H. Ralphs, and D.B. Nielsen (Ed.). *The Ecology and Economic Impact of Poisonous Plants on Livestock Production.* pp 407-424. Westview Press, Boulder, CO.
- Provenza, F.D., J.A. Pfister and C.D. Cheney. 1992. Mechanisms of learning in diet selection with reference to phytotoxicosis in herbivores. *J. Range Manage.* 45:36.
- Rhoades, D.F. 1979. Evolution of plant chemical defense against herbivores. In: G.A. Rosenthal and D.H. Janzen (Ed.). *Herbivores: Their Interaction with Secondary Plant Metabolites.* pp 3-53. Academic Press, New York.
- Schlenoff, D.H. 1984. Novelty: a basis for generalization in prey selection. *Anim. Behav.* 32:919.
- Stamm, G.W. 1975. *Veterinary Guide for Farmers.* Book Div., Hearst Corp., New York.
- Tolley, E.A., M.W. Tess, T. Johnson and K.R. Pond. 1988. Effects of switching diets on growth and digesta kinetics of cattle. *J. Anim. Sci.* 66:2551.

CHAPTER V

SYNTHESIS

Implications of Results to Plant Chemical Defenses

A vast array of plant chemicals serve as grazing deterrents (Riley and Tuck, 1985). Although these secondary compounds may serve several functions in a plant (i.e., intermediate metabolites, protection against plant pathogens, allelopathic effects on other plants, or protection against physical environmental stresses; Rhoades and Cates, 1976), the fact that many deter grazing cannot be disputed. Several ecological theories have been developed to explain the observed patterns of defensive compounds in plants (Feeny, 1976; Rhodes and Cates, 1976; Bryant et al., 1983; Coley et al., 1985). These so-called plant defense theories assume that herbivory was a major selective force in the evolution of many secondary plant compounds.

Many researchers have assessed the effect of specific phytochemicals on herbivores (Rosenthal and Janzen, 1979; Harborne, 1991), and others have assessed the physiological mechanisms of animals to mitigate the effects of secondary compounds (Freeland and Janzen, 1974; McArthur et al., 1991). However, few authors have assessed the behavioral responses of animals to specific plant chemicals (Pfister et al., 1992). And, how effective specific compounds are at deterring grazing has seldom been researched. By examining the intake response of sheep to food toxicity

in several different settings, my research can address the properties a toxin should have to be an effective grazing deterrent.

The implications of my research to herbivory apply primarily to grazing by mammalian herbivores. Learned food aversions have been demonstrated in many animals, including grasshoppers, blue jays, crows, garter snakes, tiger salamanders, bats, rats, cows, sheep, and humans (see Davey, 1989; Provenza et al., 1992). However, taste may not play the same role in food aversion learning in insects, birds, and reptiles as it does in mammals. For example, birds become averted to the sight rather than the flavor of foods (Garcia and Rusiniak, 1980). Thus, I will limit my discussion to the role of toxins in deterring mammalian herbivores.

What characteristics make a toxin an effective anti-herbivore compound? A toxin can decrease plant palatability, and therefore reduce potential herbivory, by having (1) an initially offensive flavor, (2) digestibility-reducing properties, or (3) negative post-ingestive consequences. I will discuss these properties separately but, in nature, a specific chemical may have several of these defensive attributes.

The flavor of a plant may influence the amount of material removed when the plant is initially sampled by an herbivore. Particularly offensive or strong flavors may elicit a neophobic response by animals first encountering a plant. This flavor influence was demonstrated by the avoidance of the high oregano-flavor food in the novel acceptance experiment in chapter 4. Many authors consider a repugnant flavor to be an herbivore deterrent (Rhoades and Cates, 1976; Laycock et al., 1988; Harborne, 1991; Meyer and Karasov, 1991). But, they fail to recognize that the hedonic value of a flavor can be

altered by post-ingestive consequences; a plant flavor becomes distasteful because gastro-intestinal distress follows its consumption (Molyneux and Ralphs, 1992; Provenza et al., 1992). Therefore, if the flavor of a toxin were separated from its post-ingestive consequences, flavor probably would not affect consumption except through its effect on neophobia.

Digestibility-reducing properties could also protect a plant from herbivory by decreasing positive feedback in the formation of a conditioned preference. If a secondary compound reduces the digestive pay-off of a plant, the palatability of that plant will decrease (Provenza et al., 1992). Several defense theories consider digestibility-reducing properties a major mechanism for plant defense (Rhoades and Cates, 1976; Harborne, 1991). However, I consider it a weak anti-herbivore mechanism because it would, at best, reduce the preference for a particular plant. Reduced digestibility could not lead to the complete avoidance of a plant as could occur through a conditioned aversion. Admittedly, decreasing preference may effectively prevent herbivory if alternative forages were available. When forage is limited, however, decreased preference would be unlikely to protect a plant from grazing.

The most effective means by which secondary compounds can alter plant palatability is through conditioned flavor aversions. In order to be an effective agent of a flavor aversion, a chemical must have negative post-ingestive consequences. Further, these consequences must affect the emetic system of the animal (Provenza et al., 1992). As discussed previously, mammals do not learn to avoid plants that are toxic but that do not affect the emetic system. While it is important to understand the

physiological effects of toxins on animals, it is also important to recognize that not all of these effects lead to grazing avoidance.

One factor that determines the effectiveness of a toxin in reducing palatability is how ill it makes the herbivore. My research on the effect of LiCl dose on a conditioned aversion, described in chapter 4, implies that the more ill a toxin makes an animal the more effective that toxin will be at repelling future herbivory. From a plant fitness perspective, the toxin should also be effective in small quantities (Rhoades and Cates, 1976). Although degree of illness could be altered through changes in concentration, it would be less metabolically expensive to a plant if only a small amount of a toxin caused illness.

Very little is known about what a toxin should taste like to be an effective anti-herbivore compound. However, my research does help to answer the question of whether or not a toxin should even have a detectable flavor. If a toxin has a detectable flavor, it can be selectively translocated to protect the most metabolically important plant material such as young, actively growing leaves (Rhoades, 1979). Research discussed in chapter 2 implies that herbivores can distinguish between levels of a chemical defense on the basis of flavor differences. Also, if a toxin has a distinct, detectable flavor, groups of poisonous plants might gain an anti-herbivore advantage by containing a single toxin and therefore a similar flavor, as suggested in chapter 3. My results also predict the occurrence of nutritious plants that mimic the flavor of toxic plants (chapter 3). Additionally, the more ill a toxic plant makes an herbivore the more

successful mimicry of that plant would be at deterring herbivory (generalization experiment in chapter 4).

An alternative plant strategy would be to incorporate a toxin without a detectable flavor. As demonstrated in chapter 2, if a phytochemical varies in toxicity without affecting plant flavor, the animal may form an aversion based on the maximum rather than the average toxicity. Therefore, the plant may actually gain an anti-herbivore advantage by varying the concentration of a tasteless toxin rather than maintaining a constant toxin level. Toxin variability may play an important metabolic role in plants such as toxins that are used for temporary nitrogen storage (Molyneux and Ralphs, 1992) or as substrates for useful products like amino acids or sugars (McKey, 1979). However, as suggested above, this strategy would not work for tastable toxins because the herbivore could simply adjust intake based on flavor.

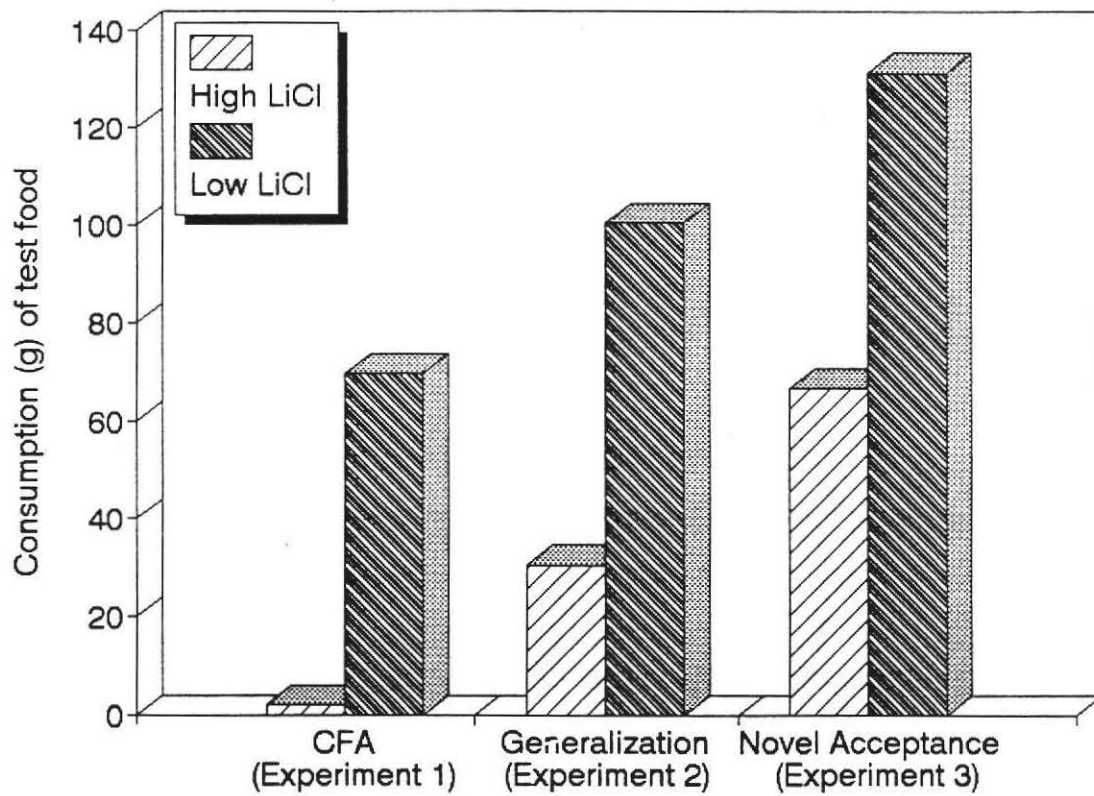
In summary, in order to understand how phytochemicals affect herbivory we must thoroughly understand the behavioral responses of animals to plant secondary chemicals. This requires knowledge of how pre-ingestive characteristics, like flavor, and post-ingestive consequences, like gastro-intestinal illness, alter palatability. Development of plant defense theories must consider herbivore characteristics in order to accurately address plant/herbivore relationships.

Literature Cited

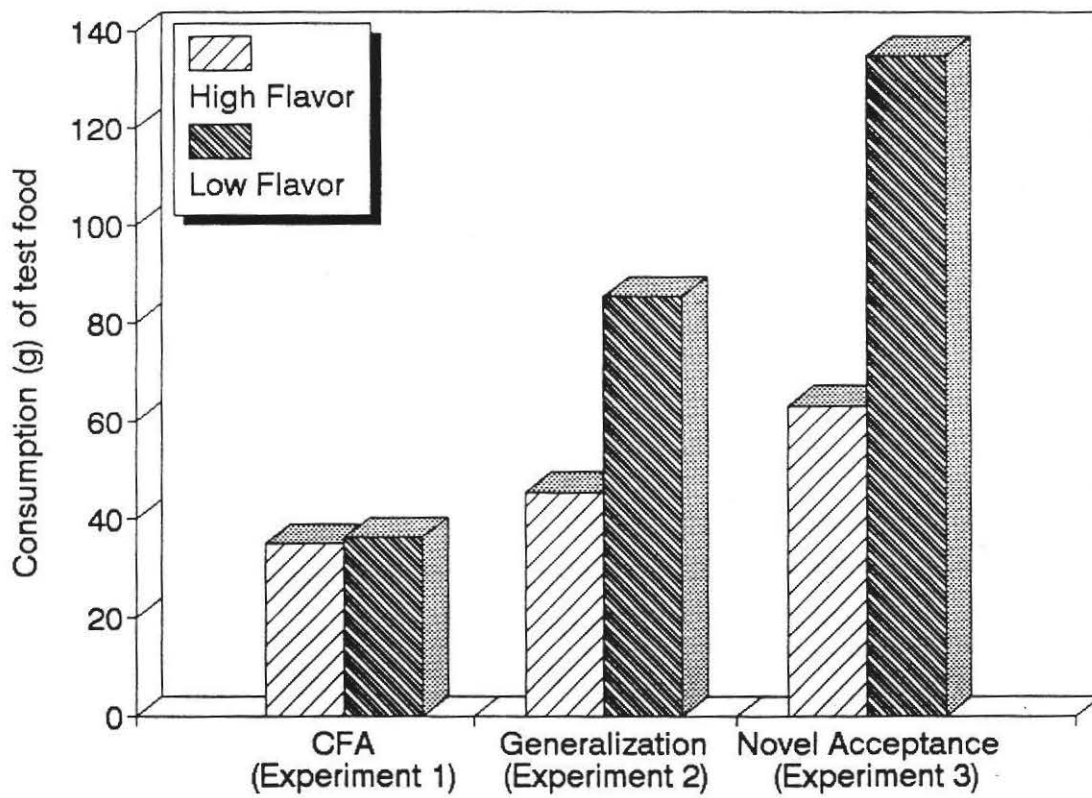
- Bryant, J.P., F.S. Chapin, III and D.R. Klein. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*. 40:357.
- Coley, P.D., J.P. Bryant and F.S. Chapin, III. 1985. Resource availability and plant antiherbivore defense. *Science* 230:895.
- Davey, G. 1989. Comparative aspects of conditioning: pavlovian learning. In: *Ecological Learning Theory*. pp 23-57. Routledge, New York.
- Feeny, P. 1976. Plant apparency and chemical defense. *Rec. Adv. Phytochem.* 10:1.
- Freeland, W.F. and D.H. Janzen 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. *Am. Nat.* 108:269.
- Garcia, J., and K.W. Rusiniak. 1980. What the nose learns from the mouth. In: D. Muller-Schwarze and R.M. Silverstein (Ed.) *Chemical Signals: Vertebrates and Aquatic Invertebrates*. pp 141-156. Plenum Press, New York.
- Harborne, J.B. 1991. The chemical basis of plant defense. In: R.T. Palo and C.T. Robbins (Ed.) *Plant Defenses Against Mammalian Herbivory*. pp 45-59. CRC Press Inc., Boca Raton, FL.
- Laycock, W.A., J.A. Young and D.N. Ueckert. 1988. Ecological status of poisonous plants on rangelands. In: L.F. James, M.H. Ralphs, and D.B. Nielson (Ed.) *The Ecology and Economic Impact of Poisonous Plants on Livestock Production*. pp 27-42. Westview Press, Boulder, CO.
- McArthur, C., A.E. Hagerman and C.T. Robbins. 1991. Physiological strategies of mammalian herbivores against plant defenses. In: R.T. Palo and C.T. Robbins (Ed.) *Plant Defenses Against Mammalian Herbivory*. pp 103-114. CRC Press Inc., Boca Raton, FL.
- McKey, D. 1979. The distribution of secondary compounds within plants. In: G.A. Rosenthal and D.H. Janzen (Ed.) *Herbivores: Their Interactions with Secondary Plant Metabolites*. pp 56-133. Academic Press, New York.
- Meyer, M.W. and W.H. Karasov. 1991. Chemical aspects of herbivory in arid and semiarid habitats. In: R.T. Palo and C.T. Robbins (Ed.) *Plant Defenses Against Mammalian Herbivory*. pp 167-187. CRC Press Inc., Boca Raton, FL.

- Molyneux, R.J. and M.H. Ralphs. 1992. Plant toxins and palatability to herbivores. *J. Range Manage.* 45:13.
- Pfister, J.A., C.D. Cheney and F.D. Provenza. 1992. Behavioral toxicology of livestock ingesting plant toxins. *J. Range Manage.* 45:30.
- Provenza, F.D., J.A. Pfister and C.D. Cheney. 1992. Mechanisms of learning in diet selection with reference to phytotoxicosis in herbivores. *J. Range Manage.* 45:36.
- Rhoades, D.F. 1979. Evolution of plant chemical defense against herbivores. In: G.A. Rosenthal and D.H. Janzen (Ed.) *Herbivores: Their Interactions with Secondary Plant Metabolites.* pp 3-53. Academic Press, New York.
- Rhoades, D.F. and R.G. Cates. 1976. Toward a general theory of plant antiherbivore chemistry. *Rec. Adv. Phytochem.* 10:168-213.
- Riley, L. and D.L. Tuck. 1985. Conditioned taste aversion: a behavioral index of toxicity. In: N.S. Braveman and P. Bronstein (Ed.) *Experimental Assessments of Clinical Applications of Conditioned Food Aversions.* pp 272-292 Vol. 443. New York Acad. of Sci.
- Rosenthal, G.A. and D.H. Janzen (Ed.) 1979. *Herbivores: Their Interactions with Secondary Plant Metabolites.* Academic Press, New York.

APPENDIX



Appendix Figure 1. The influence of LiCl dose on the intake of the test foods on the second test day in experiments 1, 2, and 3. Each bar represents mean consumption of animals receiving the same toxin dose averaged over flavor level. A high LiCl dose was 225 mg/kg and a low dose was 100 mg/kg of LiCl.



Appendix Figure 2. The influence of flavor level on the intake of the test foods on the second test day in experiments 1, 2, and 3. Each bar represents mean consumption of animals receiving the same flavor level averaged over toxin dose. A high level of flavor was 3% ground oregano and a low flavor level was 1% ground oregano.

VITA

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Education: 1980, High School Diploma, Beach High School, Beach, North Dakota; 1984, B.S. degree in Range Management, North Dakota State University, Fargo, North Dakota; M.S. degree in Range Science, Texas A & M University, College Station, Texas; Ph.D. degree in Range Science, Utah State University, Logan, Utah.

Professional Experience: 1982-1983, summer Research Technician on range science projects for the North Dakota Agricultural Experiment Station; 1984, seasonal, interpretive Park Ranger for Theodore Roosevelt National Park, Medora, North Dakota; 1985-1987, Research Assistant on grazing research at Texas A & M University, College Station, Texas; 1987-1988, Research Associate in range science for the Texas Agricultural Experiment Station, Uvalde, Texas; 1988-present, Research Assistant on mechanisms of diet selection for Utah State University, Logan, Utah.