

ROLE OF DETOXIFICATION OF PLANT  
SECONDARY COMPOUNDS ON DIET BREADTH  
IN A MAMMALIAN HERBIVORE, *Trichosurus vulpecula*

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**Abstract**—Theory predicts that mammalian herbivores detoxify different classes of plant secondary compounds via separate metabolic pathways and that generalist herbivores maintain broad diet breadth to avoid overloading individual detoxification pathways. We tested the hypothesis that a generalist marsupial herbivore, the common brushtail possum, *Trichosurus vulpecula*, can maintain a higher intake of food when allowed to select from two diets containing different profiles of secondary compounds (phenolics and terpenes) than when given access to the diets individually. Diets consisted of a fruit and vegetable mash to which was added ground leaves of either *Eucalyptus melliodora* or *E. radiata*. *E. melliodora* and *E. radiata* differ in their concentrations and types of secondary compounds. Brushtail possums include these eucalypt species as part of their natural diet. We measured food consumption and detoxification metabolites of possums on these diets. Consistent with the hypothesis, animals presented with a choice of both diets consumed more food than animals given diets singly. One of the two indicators of detoxification, acid load in urine, differed significantly between diets while the other, glucuronic acid, did not. These results provide partial support for the hypothesis that diet breadth is governed by detoxification abilities.

**Key Words**—Diet breadth, herbivory, phenolics, terpenes, *Trichosurus vulpecula*, detoxification.

INTRODUCTION

In their seminal paper on feeding strategies of herbivorous mammals, Freeland and Janzen (1974) proposed that the majority of mammalian herbivores were

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obligate dietary generalists due to limitations of their detoxification systems. Mammals possess several distinct detoxification pathways that individually process particular classes of compounds, although some redundancy exists (Sipes and Gandolfi, 1986). Freeland and Janzen (1974) predicted that the large quantities of similar plant secondary metabolites ingested from a diet of a single species of plant should exceed the capacities of the one or two detoxification pathways specific to those compounds. They predicted that mammalian herbivores could circumvent overloading any one pathway by feeding on a wide variety of plants, each containing different types of secondary metabolites that would be processed through distinct detoxification pathways.

In the 25 years since Freeland and Janzen's (1974) paper, many of the secondary metabolites occurring in plants (e.g., Adams et al., 1981; Bryant et al., 1983; Reichardt et al., 1990; Sinclair et al., 1988) as well as enzymes of the mammalian detoxification system have been described at the molecular level (e.g., Dutton, 1980; Jakoby, 1980; Sipes and Gandolfi, 1986; Testa and Jenner, 1981). Myriad studies conducted on the diets of herbivorous mammals support the assumption that most mammalian herbivores are generalist feeders (e.g., Cameron and Rainey, 1972; Dearing, 1996; Dial, 1988; Freeland, 1991; Ganzhorn, 1988; Kerle, 1984). In laboratory studies, mammalian herbivores select a diet of several plant species even when presented with ample quantities of their most preferred food types (Atsatt and Ingram, 1983; Freeland and Winter, 1975; Dearing unpublished data). Moreover, food intake is enhanced when they are presented with more than one type of food (Freeland and Winter, 1975). These lines of evidence suggest that the generalist feeding behavior of mammalian herbivores is imposed by limitations of the detoxification system; however, this hypothesis has not been tested experimentally.

An alternative hypothesis is that no single species of plant can provide all of the necessary nutrient requirements, and thus, mammals must consume a number of species to obtain a nutritionally adequate diet (Westoby, 1978). Whereas gross energy content does not vary greatly among many plant species, other variables, e.g., fiber and nitrogen, may vary tremendously between and within species as well as between different plant tissue types (Dearing and Schall, 1992). Mammals have demonstrated abilities to discriminate differences in such variables. For example, the eastern woodrat, *Neotoma floridana*, discriminates between diets that are 17.5% and 14% protein, preferring the former (Post, 1993).

We tested Freeland and Janzen's (1974) hypothesis of detoxification limitation by using a wild herbivore fed natural foods and monitoring fluxes through detoxification pathways. The system consisted of the brushtail possum, *Trichosurus vulpecula*, and two species of *Eucalyptus*, *E. melliodora* and *E. radiata*. Brushtails are common to the forests of eastern Australia and are typical generalist herbivores in that individual possums select a wide range of plant species

for their diets. In many habitats, *Eucalyptus* species comprise significant portions of their diets (Freeland and Winter, 1975; Kerle, 1984).

The two eucalypt species used in the experiment produce different types of secondary chemicals that are potentially processed through different detoxification pathways. *E. radiata* is noted for its high levels of terpene oils, which in some chemotypes approach 8% of the dry mass (Foley, 1992). Based on data in the literature, we predicted that the compounds in *E. radiata* would be detoxified primarily through the phase 1 pathway (also known as functionalization). In the phase 1 pathway, xenobiotics are made more hydrophilic and polar through oxidation, reduction, or hydrolysis, and hence easier to excrete (Sipes and Gandolfi, 1986). Many terpenes have been shown to be oxidized after ingestion (McLean et al., 1993; Southwell et al., 1980). Because the metabolites produced from the phase 1 pathway are typically unconjugated organic acids (McLean et al., 1993), urine pH and titratable acids in the urine can be used as indicators of detoxification (Foley and Hume, 1987; Foley et al., 1995).

*E. melliodora* also has terpenes; however, they typically occur in lower concentrations (1–2% of dry mass) with cineole and  $\alpha$ -phellandrene being the dominant components (Edwards et al., 1993). *E. melliodora* is reported to contain significant levels of phenolics, particularly condensed tannins (Foley and Hume, 1987). Although absorption of condensed tannins by the gut is controversial, some data suggest that condensed tannins are absorbed and require detoxification (Clausen et al., 1990; Distel and Provenza, 1991; Harmand and Blanquet, 1978). The phenolics of *E. melliodora* have not been examined in as much molecular detail as have terpenes; thus, our investigation of them was restricted to the class level. Many phenolics appear to be detoxified through phase 2 pathways (Sipes and Gandolfi, 1986). In phase 2 pathways, xenobiotics are conjugated with endogenous molecules that are hydrophilic. The primary phase 2 pathway employed by marsupials is the glucuronic acid pathway (Baudinette et al., 1980).

Although *E. radiata* and *E. melliodora* differ in their profiles of secondary compounds, they are reported to have similar nitrogen, energy, and fiber contents (Foley and Hume, 1987). This similarity allowed us to test the hypothesis of detoxification limitation while holding nutrient levels relatively constant.

Using this study system, we tested the following predictions generated from the detoxification-limitation hypothesis: (1) food intake should be greater when possums are given both *Eucalyptus* diets simultaneously than when they are given either diet individually; (2) the types or amounts of detoxification products excreted by possums should differ between *Eucalyptus* treatments; (3) when feeding on both diets simultaneously, concentrations of detoxification products excreted in the urine should be intermediate to those produced when animals are feeding on diets individually.

Here we provide the first experimental evidence that supports the hypothesis that limitations of the detoxification system impose a generalist feeding strategy on mammalian herbivores.

#### METHODS AND MATERIALS

##### *Collection and Treatment of Leaf Material*

We collected mature leaves from several trees (5–7 individuals per species) of *Eucalyptus radiata* and *E. melliodora* from forests near Tidbinbillia, Australian Capital Territory, Australia. Leaves were collected simultaneously to minimize any possible seasonal differences in chemistry. They were stored in a freezer for a week prior to being freeze-dried. Freeze-dried leaves of each species were ground in a Wiley mill through a 1-mm mesh and homogenized. They were analyzed colorimetrically in duplicate for total phenolics (Singleton and Rossi, 1965) and condensed tannins (HCl–butanol method) (Porter et al., 1986). The standards used in these assays were tannic acid for the total phenolics assay and quebracho for the condensed tannins assay. Terpenes were identified and quantified by gas chromatography with tridecane as an internal standard (Edwards et al., 1993). To verify that the leaves used did not differ in nutritional content, we measured total nitrogen (Kjeldahl assay) and fiber (acid detergent analysis) (Goering and Van Soest, 1970) in duplicate.

##### *Animals*

Six adult possums were trapped from woodlands on the property of the CSIRO's Division of Wildlife and Ecology in Canberra, Australia. Because many of the females had pouch or back young, we restricted our study to males. Possums ranged in weight from 2.44 to 3.38 kg ( $x = 2.77$ , SE = 0.12 kg), and they were kept in wire cages (1 m × 1 m × 1 m) with a nest box and wire mesh bottoms. Water was provided ad libitum.

##### *Diets*

Possums were fed a maintenance diet of dry ground alfalfa (*Medicago sativa*), fresh *Beta vulgaris* cicla (Swiss chard or silver beet), carrots, apples, sugar, and ground *Eucalyptus* leaves (Table 1). *Eucalyptus* leaves were included in the maintenance diet so that possums would maintain the same level of inducible detoxification enzymes prior to and between treatments. All diet components were finely chopped in a food processor to produce particle sizes small enough to prevent selective foraging. Possums maintained weight on the maintenance diet.

Treatment diets consisted of the maintenance diet with alfalfa, Swiss chard,

TABLE 1. COMPONENTS OF MAINTENANCE AND TREATMENT DIETS<sup>a</sup>

Maintenance diet	Treatment diet
35% apple	34% apple
6% carrot	6% carrot
10% sugar	10% sugar
25% alfalfa	50% <i>Eucalyptus</i> sp.
14% <i>B. vulgaris</i>	
5% <i>E. melliodora</i>	
5% <i>E. radiata</i>	

<sup>a</sup>All components are expressed on a dry matter basis. For the treatment diets, the *Eucalyptus* added was either *E. radiata* or *E. melliodora*.

and *Eucalyptus* components replaced with freeze-dried ground leaves of either *E. radiata* or *E. melliodora*. We chose this approach over that of feeding fresh leaves because of the enormous heterogeneity that may occur among leaves both within and among trees. We conducted preliminary feeding trials to determine which concentrations of *Eucalyptus* leaves would depress food intake to significantly less than maintenance without being completely deterrent. This tactic was necessary to ensure that when possums were presented with both treatments, they would eat from both and not only their preferred treatment. Fifty percent of each treatment diet (dry weight) consisted of either *Eucalyptus radiata* or *E. melliodora*. Each possum received all of the following treatments: *E. radiata* diet only (405 g wet weight/day), *E. melliodora* diet only (405 g wet weight/day), and a combination of both (405 g/day of each treatment). The quantity of the diet treatments offered singly, i.e., 405 g, was in excess of what possums require to maintain weight and was approximately 2× more than possums voluntarily consumed during preliminary trials. For the combination treatment, *E. radiata* and *E. melliodora* were presented in separate dishes placed side by side. This presentation allowed possums to select the amount of each treatment. Treatments were fed to each animal for five consecutive days followed by five days on the maintenance diet prior to the next treatment. Diets were prepared fresh each day. Leftovers were removed daily and dried before weighing. To account for possible effects of diet sequence, we used a crossover design where different animals received the various treatments in different orders.

#### Data Collection and Treatment

Animals were weighed at the start and end of each treatment. Food intake, urine volume, total glucuronic acid production (Blumenkrantz and Asboe-Hansen, 1973), and urine pH were measured daily. Urine was collected at room temperature in 500-ml flasks under each cage. Urine pH was measured with a

pH meter on the initial sample collected at 24°C. Titratable acids in the urine were measured during the last two days of each treatment. Twenty milliliters of urine at 24°C were titrated to pH 6.6 with 0.2 N KOH.

We compared the percent change in body mass from the start of each treatment to the end of each treatment by using repeated measures analysis of variance (ANOVA) and Tukey's HSD for differences among diets. To compare the effects of diet treatment on food intake, urine pH, titratable acid, and glucuronic acid concentration, we averaged data from the last two days of the treatment for each possum. We used this approach to remove the effects of acclimation to the diet, which may have occurred during the initial days and to allow for maximal induction of detoxification enzymes (Sipes and Gandolfi, 1986). Results were analyzed by repeated measures ANOVAs and Tukey's HSD for differences between diets. Titratable acid and glucuronic acid concentrations were not normally distributed and were transformed (square root-arcsin and natural log, respectively) prior to analysis. We performed correlations on amount consumed of diets fed individually, indicators of detoxification (glucuronic acid and pH), and number of days on each diet to identify potential relationships among these factors.

## RESULTS

There was little difference in the nutritional contents of *E. melliadora* and *E. radiata*. Both had similar concentrations of fiber (26.0% vs. 24.1% res. (Acid Detergent Fiber) ADF,  $P > 0.05$ ,  $N = 4$ ). *E. radiata* had slightly but significantly higher levels of nitrogen compared to *E. melliadora* (1.5% vs. 1.39%,  $P = 0.05$ ,  $N = 4$ ).

*E. radiata* and *E. melliadora* differed in their secondary compound profiles. *E. radiata* contained significantly higher levels of total phenolics ( $240.5 \pm 5.8$  vs  $188.8 \pm 8.5$  mg/g tannic acid equivalent,  $P < 0.05$ ,  $N = 4$ ) and equal levels of condensed tannins compared to *E. melliadora* ( $354 \pm 11$  vs.  $347 \pm 0.4$  mg/g quebracho equivalents,  $P > 0.05$ ,  $N = 4$ ). *E. radiata* had higher total concentrations of terpenes compared to *E. melliadora* (2.08% vs. 1.28% oil/dry mass, respectively). The prominent terpenes in *E. radiata* were sesquiterpenes and oxygenated monoterpenes, whereas cineole comprised the majority of the terpene spectrum of *E. melliadora* (Figure 1). Statistical comparisons of terpene content are unavailable as only one pooled sample of each species was assayed for terpenes.

Possums lost weight during all diet treatments (Table 2). The greatest change in body mass occurred when they were feeding on the *E. radiata*-only treatment; there was no significant difference in weight loss of possums feeding on *E. melliadora*-only and both diets (Table 2). Possums recovered all weight lost on treatments during the five-day rest period.

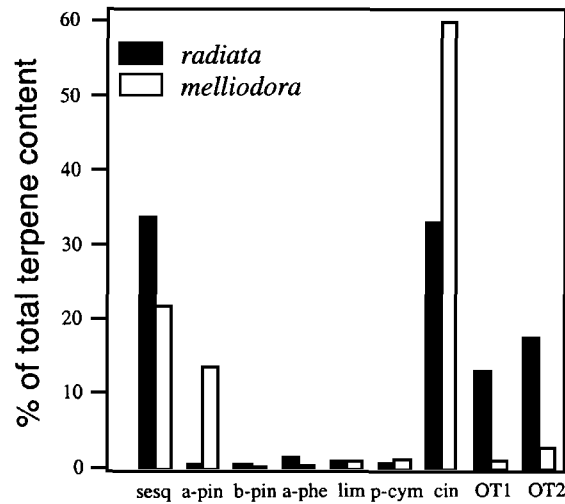


FIG. 1. Secondary chemistry of *Eucalyptus melliodora* and *E. radiata*. Terpene abbreviations are: sesq, sesquiterpenes; a-pin,  $\alpha$ -pinene; b-pin,  $\beta$ -pinene; a-phe,  $\alpha$ -phellandrene; lim, limonene; p-cym, paracymene; cin, cineole; OT1, uncharacterized oxygenated terpene 1; OT2, uncharacterized oxygenated terpene 2.

The amount of food consumed varied significantly with treatment (Figure 2, Table 3). Possums consumed 33% more food when offered both diets simultaneously compared to either diet offered singly. When offered both diets simultaneously, possums ate significantly less of each diet than when presented singly. However, this difference was statistically significant for only the *E. radiata* treatment (*E. radiata*:  $t = 3.15$ ,  $df = 5$ ,  $P = 0.025$ ; *E. melliodora*:  $t = 2.21$ ,  $df = 5$ ,  $P = 0.07$ ). In none of the treatments did possums achieve food intake levels comparable to that of the maintenance diet, approximately 40 g dry matter/kg<sup>0.75</sup>/day. Regardless of treatment, they never consumed all of any one treatment offered in a 24-hr period, i.e., leftovers were always present.

TABLE 2. MEAN PERCENT WEIGHT CHANGE OF POSSUMS AFTER EACH DIET TREATMENT<sup>a</sup>

Diet treatment	Weight change
<i>E. melliodora</i> only	-4.4 (0.7) a
<i>E. radiata</i> only	-6.5 (1.1) b
Both	-3.1 (0.6) a

<sup>a</sup>Standard errors are given in parentheses. Changes in weight with different letters indicate significant differences (Tukey's HSD).

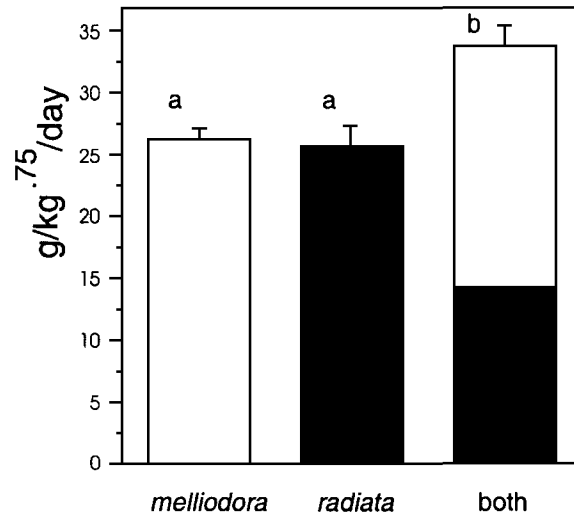


FIG. 2. Average food intake (+ 1 SE) in grams for the last two days on each diet treatment. Intake was standardized for metabolic body mass. Intakes with different superscripts are significantly different. The open segment of "both" represents the amount of *E. melliodora* consumed when both diets were presented simultaneously, the filled segment represents the amount of *E. radiata* consumed.

TABLE 3. SUMMARY OF REPEATED MEASURE ANOVAS<sup>a</sup>

	<i>F</i> ratio	<i>P</i>
% Change mass		
Within subjects		
Diet	5.44	<b>0.025</b>
Food intake		
Within subjects		
Diet	26.9	<0.001
Glucuronic acid		
Within subjects		
Diet	1.33	0.31
pH		
Within subjects		
Diet	34.0	<0.001
Titrateable acid		
Within subjects		
Diet	17.8	<0.001

<sup>a</sup>Degrees of freedom were 2, 10 for all analyses. Significant results are in bold.



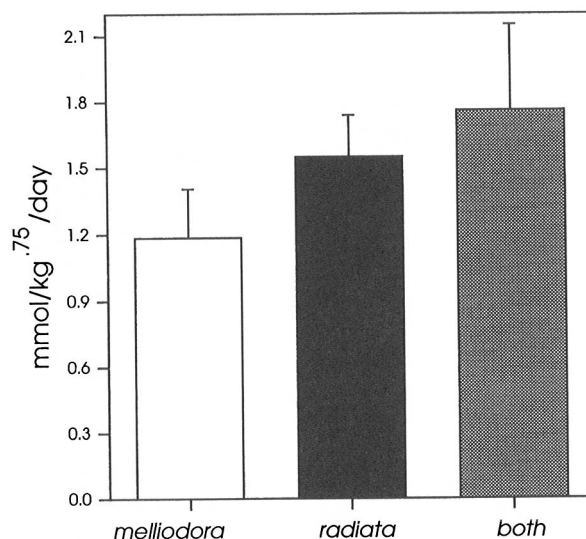


FIG. 3. Mean (+ 1 SE) concentration of glucuronic acid in urine averaged for the last two days of each diet treatment.

The excretion of detoxification metabolites in urine was dependent on both diet treatment and detoxification pathway. There was no difference in the total amount of glucuronic acid excreted on different diets (Figure 3, Table 3). There were differences in urine pH between treatments (Figure 4, Table 3). Animals consuming the *E. radiata* diet produced urine with the lowest pH, possums given both diets produced urine of intermediate pH, and those consuming the *E. melliodora* produced urine with the highest pH (Figure 4). The concentration of titratable acids was significantly greater in the urine of the possums on the *E. radiata* treatment. There was no significant difference in titratable acid levels in animals on the *E. melliodora* and both treatments (Figure 4, Table 3).

Within the diet treatments of *E. radiata* and *E. melliodora* offered singly, daily glucuronic acid production and urine pH were not correlated with daily food intake (Table 4). However, the number of days on each treatment and urine pH were negatively correlated (Table 4). This correlation was most pronounced for animals consuming the *E. radiata* diet. In contrast, glucuronic acid concentrations were not correlated with number of days on the treatment.

#### DISCUSSION

Freeland and Janzen (1974) hypothesized that mammalian herbivores are forced to be generalist foragers due to their inability to detoxify large quan-

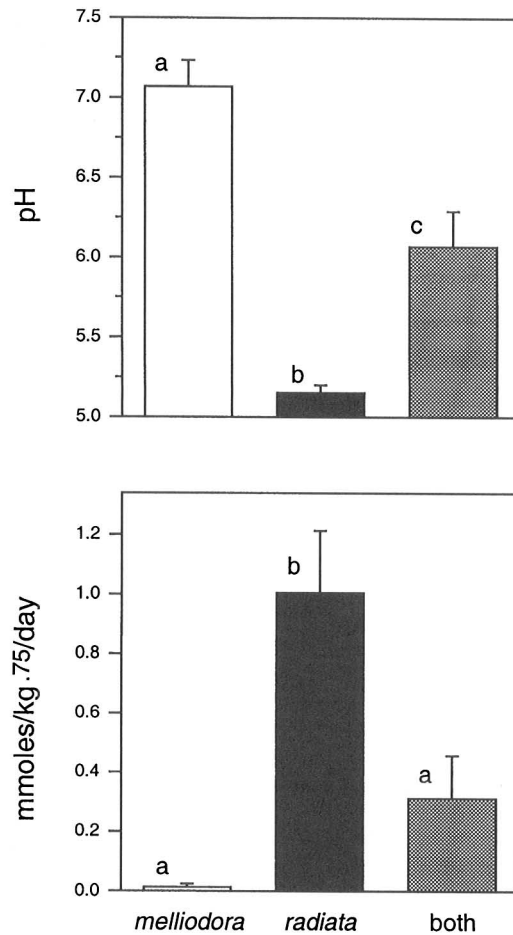


FIG. 4. pH and titratable acids in the urine. The top panel shows urine pH averaged over the last two days of each treatment. The lower panel shows the amount of titratable acids in the urine averaged for the last two days of each treatment. Within a panel, bars with different letters are significantly different. Error bars are one standard error.

tities of similar toxins. Our results provide partial support for this hypothesis. In accordance with the hypothesis, possums ate more food, approximately 33% more, when offered different diets simultaneously versus singly. Production of detoxification metabolites varied as predicted in one (titratable acids) of the two indicators of detoxification. Possums excreted urine with the greatest amounts of titratable acids when consuming *E. radiata* diet, intermediate amounts when feeding on both diets, and the lowest amounts when feeding on *E. melliodora*.

TABLE 4. CORRELATION MATRIX OF FOOD INTAKE, NUMBER OF DAYS A TREATMENT, AND INDICATORS OF DETOXIFICATION FOR POSSUMS CONSUMING *E. melliodora* OR *E. radiata* DIET<sup>a</sup>

	Food intake	Days ( <i>N</i> )
<i>E. melliodora</i>		
GA	-0.14	0.29
pH	0.35	-0.67*
Food Intake		-0.621*
<i>E. radiata</i>		
GA	0.40	0.369
pH	-0.178	-0.901*
Food Intake		0.11

<sup>a</sup>Significant correlations are marked with asterisks. GA = glucuronic acid production. Because of the multiple comparisons, correlations were not considered significant unless  $P < 0.006$ .

There were no differences in the excretion of glucuronic acid among possums on the different treatments.

Freeland and Janzen (1974) predicted that when an herbivore consumed the maximum quantity of a particular toxin, individual detoxification pathways became saturated, resulting in negative feedback on food intake. More recently it has been suggested that the elimination routes of detoxification products become saturated and result in a similar negative feedback (Foley, 1992). In particular, excretion of high concentrations of acidic detoxification metabolites in the urine, comparable to those found in possums consuming the *E. radiata* diet in this study, conceivably place an upper limit on the amount of the acid-generating food that can be ingested (Foley, 1992). Possums eliminate terpenes, such as those in *E. radiata*, typically through oxidative processes (Southwell et al., 1980). This reaction yields products that are strong organic acids (Foley, 1992; Foley et al., 1995; McLean et al., 1993). The mammalian kidney has a finite ability for concentrating urine; maximal concentration levels are approximately three orders of magnitude from blood concentrations (Foley et al., 1995). In units of pH, the kidney should approach its maximal concentrating ability at approximately 4.5 (Foley and McArthur, 1994; Foley et al., 1995). After five days on the *E. radiata* diet, possums produced urine with a pH that approached this lower physiological limit. In addition to the difficulties of excreting an extremely acidic urine, metabolic acidosis presents other severe physiological consequences, such as mineral loss from the skeletal system and increased nitrogen loss (Foley and McArthur, 1994; Foley et al., 1995).

The production of acidic detoxification metabolites may have cumulative effects in the long term. During the five-day course of the *E. radiata* treatment, the pH of the urine decreased progressively and dramatically while food intake remained constant. These results suggest a declining ability of possums

to buffer organic acids produced during detoxification. Thus, as buffering stores were depleted, urine pH dropped. Since by the end of day 5 urine pH approached the minimum pH possible, longer term exposure to the *E. radiata* diet may have resulted in a reduction in food intake.

Contrary to our prediction, levels of glucuronic acid in the urine did not differ significantly between possums on the three treatments. Our initial decision to use *E. radiata* and *E. melliadora* was based on previously reported levels of phenolics and terpenes (Foley, 1992; Foley and Hume, 1987). *E. radiata* was reported to have higher terpene concentrations than *E. melliadora*, as we confirmed, and approximately half the concentration of phenolics found in *E. melliadora* (Foley and Hume, 1987). Thus, we had expected that the *E. melliadora* treatment would have phenolic levels double those of the *E. radiata* treatment. Unfortunately, the leaves of *E. radiata* that we used had unusually high concentrations of total phenolics, significantly more than the *E. melliadora* leaves and more than twice the amount reported in the literature (Foley, 1992; Foley and Hume, 1987). In addition, both *E. melliadora* and *E. radiata* had similar high levels of condensed tannins, a subclass of phenolics. Based on intake and phenolic levels of each diet, there were no differences in doses (milligrams of phenolics consumed per day) of either total phenolics or condensed tannins on either diet treatment alone. Therefore, we would not expect to find a difference in glucuronic acid excretion between the two treatments alone. Possums ingested greater doses of total phenolics (15% more) and condensed tannins (22% more) when consuming both diets simultaneously compared with diets fed singly. Although there was no significant statistical difference in glucuronic acid excretion, on both diets, glucuronic acid excretion was 48% higher in possums consuming both treatments compared with *E. melliadora* alone and 14% higher on both diets compared with *E. radiata* alone (Figure 3). The large variation in glucuronic acid excretion between individuals may have hindered our ability to detect differences.

Metabolites of both the phase 1 and phase 2 pathways contribute to the total titratable acid in the urine. Although we did not identify individual detoxification metabolites, we credit the high concentration of titratable acid in the urine of possums on the *E. radiata* treatment to the phase 1 pathway based on the following evidence. The predominant phase 2 pathway in marsupials is the glucuronic acid pathway (Baudinette et al., 1980). Levels of glucuronic acid did not differ tremendously among possums on the various diet treatments and thus cannot account for the large increases in levels of titratable acid in animals on the *E. radiata* treatment. Moreover, investigations of marsupials consuming either individual terpenes found in *E. radiata* or entire leaves of *E. radiata* have demonstrated that terpenes are detoxified through the phase 1 pathway (McLean et al., 1993; Southwell et al., 1980).

Is there evidence that either detoxification route (phase 1 or phase 2 glucuronidation) measured in this experiment became saturated? Our data suggest that

when possums were consuming the *E. radiata* diet alone, phase 1 detoxification may have been limited by the generation and elimination of acidic detoxification metabolites. On this diet, urine pH approached the minimum pH that mammals can excrete (Foley and McArthur, 1994; Foley et al., 1995). Detoxification limitations of possums on the *E. melliodora* and both diet treatments are more difficult to attribute to either pathway measured here. Assuming that there was no change in glucuronic acid production on the three treatments, as suggested by the statistical analysis, it is plausible that the glucuronic acid pathway was saturated on all three diets. The physiological limits of glucuronic acid production by possums is unknown. Therefore, it is impossible to say whether the amount of glucuronic acid produced was at saturation levels. Lastly, mammals possess conjugation pathways other than the glucuronic acid pathway. We chose to focus on the glucuronidation pathway because it is the predominant one in marsupials (Baudinette et al., 1980). We cannot exclude the possibility that a phase 2 pathway other than the glucuronic acid was saturated and responsible for limiting food intake.

The quantity of *Eucalyptus* leaves in the treatment diets prevented possums from maintaining body mass. Thus, they represent potential maximal amounts of each eucalypt species that possums can tolerate. In nature, when food items other than *Eucalyptus* are available, the amount of *Eucalyptus* consumed may be considerably less than that consumed in this study. Wild possums tend to consume a variety of food items each night (Freeland and Winter, 1975). The incorporation of many food items in the diet, particularly those lower in secondary compounds than *Eucalyptus*, e.g., grasses, forbs, fruits (Fitzgerald, 1978; Kerle, 1984), may substantially reduce impacts, especially long-term ones, to the detoxification system. Such a foraging strategy may act to maximize the net nutritional content extracted from the diet while simultaneously minimizing costs of detoxification.

To test the detoxification-limitations hypothesis without the potentially confounding effects of differences in nutrient content, we attempted to hold nutrient levels constant among treatments by using species of eucalypts with similar nutritional contents. Fiber did not differ between the two species. Nitrogen differed slightly but significantly between leaves of the two species. However, the difference in nitrogen content does not invalidate our conclusions. *Eucalyptus* leaf comprised 50% of each diet treatment (Table 1), thus, the difference in nitrogen content between the *E. radiata* and *E. melliodora* treatments was 0.06%. We do not know whether possums can discriminate between diets differing at these nitrogen levels. We found no evidence in our experiments that they did. Although the *E. radiata* treatment was slightly higher in nitrogen, possums consumed equal amounts of the *E. radiata* or *E. melliodora* treatments when offered singly. Moreover, on all treatments, food intake was below maintenance intake, as evidenced by weight loss. This result suggests that food intake was not being regulated by nitrogen intake but rather was being limited by inhibiting agents, such as plant secondary compounds.

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