Nitrogen and Energy Retention and Acid-Base Status in the Common Ringtail Possum (*Pseudocheirus peregrinus*): Evidence of the Effects of Absorbed Allelochemicals

William J. Foley*
Department of Ecology and Evolutionary Biology, Monash University, Clayton 3168, Australia

Accepted 8/30/91

Abstract

*Common ringtail possums (*Pseudocheirus peregrinus*) were fed* Eucalyptus radiata *or* Eucalyptus ovata *foliage or an artificial diet, and parameters of acid-base status and nitrogen and energy retention were measured.* Eucalyptus radiata *foliage was more digestible than* E. ovata *foliage. This was consistent with differences in the fiber and lignin content of the diets. The higher terpene content of* E. radiata *leaf meant that digestible energy (DE) intakes were higher than on the other diets, but most of this energy was lost in the urine since metabolizable energy was only 60% of DE on the* E. radiata *diet but 85%–90% on the other diets. Animals fed* E. radiata *produced acid urines (pH 5.7), whereas other diets led to alkaline urine. The acid urines contained more titratable acid and glucuronic acid and ammonium was their major form of urinary nitrogen, whereas on the other two diets urea predominated. This did not reflect differences in urea recycling, because, although the proportion of urea produced that was degraded was similar, animals fed* E. ovata *diets produced and degraded significantly greater amounts of urea than did animals fed* E. radiata. *There were no significant differences in blood pH or* P_{CO_2} *accompanying these differences in urine composition. Animals excreted acid urine within 7 h of eating* E. radiata *leaf, and changes in food intake were mirrored by changes in urinary ammonium and glucuronic acid. However, urinary urea excretion and pH declined as soon as* E. radiata *was introduced into the diet and were unaffected by changes in food intake. These results were interpreted as responses to disturbances to acid-base status resulting from the detoxification of dietary allelochemicals.*

* Present address: Department of Zoology, James Cook University, Townsville 4811, Australia.

Physiological Zoology 65(2):403–421. 1992. 0031-935X/92/6502-919$02.00

Introduction

There have been many studies on the importance of plant secondary compounds or allelochemicals to feeding and digestion in mammalian herbivores. To date, this effort has largely been directed toward assessing correlations between gross food preferences and particular groups of allelochemicals. Whereas several studies have shown associations between food choice and allelochemical content (e.g., Oates, Waterman, and Choo 1980; Marks et al. 1988), most of these have relied on extrapolations from in vitro or insect studies to predict the effects of allelochemicals in vertebrate herbivores. Remarkably, there have been few attempts to identify the specific effects of allelochemicals on animal metabolism in vivo or to determine the extent of their interaction with primary nutrients (Robbins et al. 1987).

Additionally, most studies have focused on the fate of allelochemicals in the digestive tract. For example, Cluff et al. (1982) showed that sagebrush terpenes had little effect on digestion in mule deer because they were rapidly removed from the gut. Rapid absorption may avoid potential interactions with food components, but absorbed allelochemicals must still be detoxified and excreted, and the costs of these processes are largely unknown. This article is concerned with the possible role that absorbed allelochemicals play in limiting nitrogen and energy retention in herbivorous marsupials.

Eucalyptus leaves are generally perceived to be well defended against herbivores, since they contain significant quantities of phenolics (up to 40% of leaf dry matter; Fox and MacCauley 1977) and terpenes (up to 22% of dry mass; Morrow and Fox 1980). However, to date there is little evidence that these allelochemicals influence food choice in either insect or mammalian herbivores (Fox and MacCauley 1977; Southwell 1978; Morrow and Fox 1980; Cork and Pahl 1984). Nonetheless, a large proportion of the allelochemicals that are ingested are absorbed from the digestive tract (Eberhard et al. 1975; Cork, Hume, and Dawson 1983; Foley, Lassak, and Brophy 1987). These must then be detoxified, primarily by conversion to acidic conjugates (Caldwell 1982), and excreted in the urine or bile, and these processes may be energetically expensive.

There are several consequences of these results. First, the urinary excretion of metabolites of phenolics and terpenes means that a large proportion of the dietary energy of *Eucalyptus* leaves is unavailable to the animal. Second, the use of nutrients such as glucose for conjugation involves a further drain on nutrients. Third, any animal that consumes diets containing absorbable allelochemicals may well be faced with the problem of disposing of the metabolically produced acids each day. There is thus good reason for in-

vestigating the metabolism of absorbed allelochemicals from a nutritional viewpoint.

The studies reported here aimed to measure the acid base and nutrient status of the common ringtail possum (*Pseudocheirus peregrinus*) when fed natural and artificial diets that differed markedly in allelochemicals. A second experiment was designed to determine the speed at which changes in urine composition and buffering occur, and a third aimed to characterize the kinetics of urea nitrogen under different acid-base states.

Material and Methods

Animals

This research was approved by the Animal Experimentation Ethics Committee of Monash University and conforms with the Australian Code of Practice for the care and use of animals for scientific purposes.

Six common ringtail possums (*Pseudocheirus peregrinus*) were captured in *Eucalyptus* woodland at Monash University and maintained in large outdoor enclosures. They were fed a mixture of *Eucalyptus radiata* and *Eucalyptus ovata* leaf and apples and bananas ad lib. After 5 wk, they were moved indoors and held in individual metabolism cages as previously described (Foley and Hume 1987). The room was maintained at 17°–23°C, and the natural light regime prevailed (≃14L:10D).

Experiment 1: Acid-Base Status on Different Diets

The experiment was designed as a combination of two Latin squares (Cochran and Cox 1957). Each period lasted 16 d, of which the first 10 d served as an adaptation period. Quantitative collections of feces and urine were made on days 11–15, and blood was sampled on day 16. The diets were then changed so that one animal of each pair received one of the other two diets. Therefore, all animals received each diet once, and the possibility of carryover effects was controlled.

The diets used were mature leaves of *E. radiata,* chosen because of its high content of terpenes (Foley et al. 1987), *E. ovata,* which is notably low in terpenes (Simmons and Parsons 1987), and, third, an artificial diet based on fruit and cereals. The foliages were collected every 5–7 d from three trees growing close to each other and stored at 4°C with the ends of branches in water. The artificial diet was prepared fresh each day and presented as a wet mash. It consisted of (in % dry matter) 31% grated apple, 31% grated whole banana, 31% WeetBix (a commercial breakfast cereal; Sanitarium

Health Foods), 6.5% casein, and 0.5% of a vitamin and mineral mix (Colborn-Dawes). Details of the chemical composition of the three diets are given in table 1.

The procedures used to sample the diets and to collect feces and urine were similar to those previously described (Foley and Hume 1987). However, in the current experiments, urine was collected into flasks containing sufficient liquid nitrogen to keep the material frozen until collection. In this way, reliable measures of urinary pH could be obtained while avoiding the potential loss of nitrogen as ammonia.

To sample blood, animals were sedated with an intramuscular injection of alphaxalone/alphadolone ("Saffan," Glaxo; 0.4 mL/kg), and capillaries on the surface of the ear were cut with a scalpel blade. One hundred microliters of blood was collected anaerobically into a heparinized capillary tube (Radiometer) containing a stainless steel mixing bar. The tube was capped to exclude air bubbles and the blood was thoroughly mixed with a magnet. The sample was stored in ice water for a maximum of 2.5 h before analysis of pH and P_{CO_2}. A second blood sample (≃1 mL) was collected from the lateral tail vein into an evacuated tube containing lithium heparin, and the plasma was separated by centrifugation. A third sample (100 μL)

TABLE 1
Composition of the diets

Diet	*Eucalyptus radiata*	*Eucalyptus ovata*	Artificial
Organic matter	96.5 ± .2	95.5 ± .1	97.0 ± .1
Total nitrogen	1.55 ± .05	1.93 ± .04	1.83 ± .03
Neutral detergent fiber	33.4 ± .4	38.6 ± .5	13.6 ± .4
Acid detergent fiber	26.8 ± .5	29.4 ± .6	10.4 ± .3
Acid lignin	8.1 ± .3	9.8 ± .7	1.1 ± .2
Gross energy (kJ/g dry matter)	22.9 ± .1	19.8 ± .4	17.1 ± .2
Terpenes	8.0 ± .3	.4 ± .0	Trace
Total extractable phenols[a]	10.1 ± .4	14.6 ± .1	.3 ± .1
PPT phenols (% of above)[b]	67 ± 3	76 ± 2	89 ± 1
Sodium (mmol/g dry matter)	.11 ± .01	.11 ± .01	.05 ± .01
Potassium (mmol/g dry matter)	.15 ± .02	.25 ± .02	.23 ± .01

Note. Values are expressed as percentage of dry matter (mean ± SE; $N = 6$) unless otherwise indicated.
[a] Total phenolics extractable in 70% aqueous acetone.
[b] Proportion of total phenolics precipitated by cinchonine sulfate.

was mixed with 0.5 mL of ice-cold perchloric acid (0.6 N), and the supernatant was collected by centrifugation.

Experiment 2: Time Course of Urine Acidification

Six common ringtail possums were held in individual metabolism cages and fed the artificial diet described above for 2 wk. The last day that the animals were fed this diet (designated day 0), measurements of dry matter intake and urine production were commenced as described. The animals were then offered *E. radiata* foliage that had been cut from a single tree (days 1–13). On day 1, urine was collected every 2 h, but at other times collections were made once per day. On day 14 the artificial diet was reintroduced, and urine was collected for a further 2 d.

The urine was later thawed, and pH and volume were measured immediately. Subsamples were later analyzed for total nitrogen, urea-N, ammonium-N, inorganic phosphorus, glucuronic acid, and titratable acidity. Titration curves were constructed for 15 random urine samples by adding small volumes of 0.2 N NaOH to the urine and recording the pH after each addition. All titrations were carried out at a constant temperature of 25°C.

Experiment 3: Urea Kinetics in Animals Fed Two Foliage Diets

Twelve common ringtail possums were randomly allocated to two diets, *E. radiata* leaf and *E. ovata* leaf. All leaves were collected from a single tree of each species. The animals were maintained in individual metabolism cages, as described, for a 2-wk adaptation period and fed foliage. After this, the experimental foliage was introduced, and days 1–5 served to ensure that food intake was stable. Significant daily variation in food intake of one animal from each treatment led to their exclusion from the experiment. For the remaining 10 animals, quantitative measurements of food intake and feces and urine output were made over the next 5 d (days 6–10) as described. On day 11, the animals were reweighed, the bladder was emptied, and they were injected with 0.185 MBq [^{14}C]-labeled urea (Amersham International). Animals were fed normally, and the urine collection apparatus was checked each hour for the next 48 h. If urine was present, the volume was measured and a clean collection apparatus attached to the cage. Further details of the procedures used are given in Chilcott and Hume (1984*b*) and Foley and Hume (1987).

Analytical

Samples of the diets offered and the food refusals were freeze-dried and feces oven-dried at 50°C prior to chemical analysis. All samples were ground to pass a 1-mm screen. The residual dry matter content was determined by drying subsamples to a constant mass at 85°C. The organic matter content was measured as the loss in mass after the sample had been burned in a muffle furnace at 550°C for 4 h.

Total nitrogen content of food, feces, and urine was measured according to a semimicro Kjeldahl technique (Kjeltec Auto 1030 analyzer: Tecator, Sweden), and the gross energy content was measured in a Gallenkemp ballistic bomb calorimeter. Urine samples were freeze-dried onto cotton wool before ignition in the bomb calorimeter, and a correction was applied for the energy content of the cotton wool. Levels of neutral detergent fiber, acid detergent fiber, and permanganate lignin in the diets were measured sequentially according to the techniques described by Goering and Van Soest (1970). All samples were extracted overnight with 70% aqueous acetone (Cork and Pahl 1984) to avoid possible interference by phenolics. The diets were assayed for sodium and potassium by flame photometry.

Terpenes were measured by steam distillation as previously described (Foley et al. 1987). Total extractable phenolics were measured in 70% aqueous acetone extracts by the Folin-Ciolcateau procedure. Nontannin phenolics were determined by the same procedure after addition of cinchonine sulfate to the aqueous acetone extracts (Cork and Pahl 1984). Gallic acid was used as a standard in both phenolic assays.

Blood pH and P_{CO_2} were determined with a Çorning blood gas analyzer. Plasma concentrations of sodium, potassium, chloride, calcium, urea, creatinine, uric acid, and total protein were measured on a Kodak Ektachem dry chemistry system. Plasma lactate was measured enzymatically (Boehringer-Mannheim), and plasma magnesium was determined by atomic absorption spectrometry.

Each urine sample was thawed, and the pH was measured with an Orion pH electrode. Urine was then bulked for each animal over the 5 d of each collection, and a subsample was acidified with glacial acetic acid so that the pH was less than 3. The remainder of the sample was stored at −20°C. Urine urea was assayed by the diacetyl monoxime method of Crocker (1967); urinary ammonium was measured directly with an ammonium electrode (Orion no. 9512). Urine sodium and potassium were determined by flame photometry, chloride with a chloridometer, and magnesium by atomic absorption spectrometry. Titratable acidity was assayed by the method of Wrong

and Davies (1959), and glucuronic acid according to the method of Blumenkrantz and Asboe-Hansen (1973).

The activity of [^{14}C] in urine was assayed by liquid scintillation counting as described by Foley and Hume (1987). All samples were acidified and were bubbled with CO_2 to ensure that none of the [^{14}C] label was present as bicarbonate.

Calculations and Statistics

The concentration of blood bicarbonate was calculated from the Henderson-Hasselbalch equation,

$$pH = pK' + \frac{\log [HCO_3^-]}{\alpha CO_2 \cdot P_{CO_2}},$$

where the constants pK′ and αCO_2 were calculated for each sample from the equations given by Heisler (1989).

Parameters of urea kinetics were calculated according to the procedures described by Cocimano and Leng (1967). Possible differences between means were assessed in experiment 1 by a repeated-measures ANOVA, and comparisons between two means in experiment 3 were made by ANOVA and ANCOVA. To facilitate comparisons between animals of different body mass, digestive parameters have been expressed in terms of metabolic body mass ($kg^{-0.75}$).

Results

Experiment 1

Intake and Excretion of Dry Matter, Nitrogen, and Energy. The dry matter intake of animals fed *Eucalyptus radiata* foliage was greater ($P < 0.01$) than animals fed the other two diets, but there was a marginal carryover effect ($P = 0.04$) from previous treatments (table 2). The higher intake of dry matter of animals fed *E. radiata* foliage was offset by a lower digestibility ($P < 0.01$), so that there was no significant difference in digestible dry matter intake between the two foliage diets.

Nitrogen intake was similar for all treatments, but fecal nitrogen was significantly lower ($P < 0.05$) on the artificial diet than on the foliage diets. This largely reflected the higher ($P < 0.01$) apparent digestibility of the nitrogen on the artificial diet. In spite of this, there were no significant differences in urinary nitrogen loss or nitrogen balance between treatments.

TABLE 2

Intake, excretion, and digestiblity of dry matter, nitrogen, and energy in common ringtail possums fed two Eucalyptus *species and an artificial diet*

Diet	*E. radiata*	*E. ovata*	Artificial
Dry matter:			
Intake	38.6 ± 1.5	31.1 ± 2.5	31.2 ± 2.9
Digestibility (%)	65.7 ± .8	72.5 ± 1.3	91.1 ± 1.7
Nitrogen:			
Intake	.60 ± .03	.60 ± .05	.57 ± .06
Feces	.17 ± .02	.15 ± .01	.06 ± .02
Urine	.41 ± .03	.38 ± .02	.38 ± .03
Balance	+.02 ± .03	+.07 ± .04	+.13 ± .03
Apparent digestibility (%)	71.6 ± 1.7	74.3 ± 1.5	90.3 ± 1.8
Energy intake ($MJ \cdot kg^{-0.75} \cdot d^{-1}$):			
Gross	.88 ± .04	.62 ± .05	.52 ± .05
Digestible	.55 ± .01	.41 ± .04	.46 ± .04
Metabolizable	.37 ± .02	.35 ± .03	.44 ± .04

Note. Values are expressed as $g \cdot kg^{-0.75} \cdot d^{-1}$ (mean ± SE; $N = 6$) unless otherwise indicated.

Gross energy intake was significantly higher ($P < 0.01$) in animals fed *E. radiata* foliage primarily as a result of the higher dry matter intakes and higher gross energy content of this diet. Again there was a marginal ($P = 0.05$) carryover effect on gross energy intake. However the higher gross energy intake of animals fed *E. radiata* was offset by higher energy losses in the excreta compared with animals fed *E. ovata* leaf. In particular, urinary energy losses were significantly higher ($P < 0.01$), and, as a consequence, metabolizable energy intake was similar on all diets.

Urine Data. Urine pH was significantly lower ($P < 0.001$) and titratable acid excretion significantly higher ($P < 0.01$) in animals fed *E. radiata* foliage than in animals on other diets (table 3). This was associated with significantly lower ($P < 0.01$) urinary urea concentrations and output and a high ($P < 0.001$) ammonium output compared with other diets, but

TABLE 3
Composition of the urine of common ringtail possums fed two Eucalyptus *species and an artificial diet*

Diet	*E. radiata*	*E. ovata*	Artificial
Volume (mL/d) ...	64.0 ± 6.1	45.8 ± 5.2	54.7 ± 4.6
pH (units)	5.73 ± .03	7.66 ± .07	7.67 ± .08
Titratable acid	2.1 ± .2	−2.8 ± .7	−3.7 ± .5
Glucuronic acid ...	1.73 ± .14	.66 ± .05	.15 ± .02
Urea-N	.99 ± .33	14.99 ± 1.20	18.08 ± 1.71
Ammonium-N	16.27 ± 1.33	1.50 ± .22	1.24 ± .15
Sodium	2.71 ± .58	1.32 ± .33	.62 ± .30
Potassium	3.48 ± .92	5.43 ± .49	5.98 ± .36
Chloride	5.01 ± 2.03	2.95 ± 1.32	2.30 ± .48
Calcium	1.68 ± .45	2.14 ± .33	.57 ± .22
Magnesium	2.28 ± .45	2.01 ± .28	.85 ± .14
Phosphate	1.22 ± .17	1.36 ± .14	1.45 ± .17

Note. Values are expressed as mmol · d^{-1} (mean ± SE; $N = 6$) unless otherwise indicated.

there was no significant difference in total urinary nitrogen excretion between the diets (table 2). On all diets, ammonium and urea accounted for at least 70% of the total urinary nitrogen. Animals fed *E. radiata* excreted significantly more ($P < 0.01$) glucuronic acid than animals on the other diets. The ratio of ammonium to glucuronic acid on diets of *E. radiata* (7.0) was similar to that on the artificial diet (6.3). However, on *E. ovata,* glucuronic acid excretion was significantly higher than on the artificial diet but ammonium excretion was similar. This pattern did not reflect differences in urinary buffering, since neither titratable acid excretion nor urine pH was significantly different between *E. ovata* and the artificial diet.

Blood Data. There were few significant differences in blood or plasma values that could be attributed to diet (table 4). In particular, blood pH and P_{CO_2} were similar on all three diets. However, calculated plasma bicarbonate was significantly lower ($P < 0.01$) on diets of *E. radiata* foliage than *E. ovata* foliage but there was no significant difference between *E. radiata* and the artificial diet. Plasma urea concentrations were significantly lower ($P < 0.05$) in those animals fed *E. radiata* foliage

TABLE 4

Composition of the blood of common ringtail possums fed two Eucalyptus *species and an artificial diet*

Diet	*E. radiata*	*E. ovata*	Artificial
pH	7.384 ± .016	7.383 ± .029	7.347 ± .021
P_{CO_2} (Torr)	42.5 ± 1.6	47.8 ± 3.9	47.7 ± 4.1
HCO_3^- (mM)	24.8 ± .9	27.6 ± .9	25.1 ± .9
Urea-N (mM)	1.5 ± .5	4.1 ± .2	4.1 ± .5
Creatinine (μM)	41.5 ± 4.4	39.7 ± 2.3	61.5 ± 10.8
Uric acid (μM)	20.5 ± 1.1	22.2 ± 1.9	28.0 ± 1.9
Lactic acid (mM)	2.8 ± .5	1.8 ± .4	3.0 ± .5
Total protein (g/L)	59.0 ± 1.9	60.7 ± 1.1	57.1 ± 2.4
Electrolytes (mM):			
Sodium	140 ± .4	141 ± .4	140 ± .6
Potassium	4.83 ± .17	4.87 ± .25	4.65 ± .20
Chloride	103 ± 1.2	100 ± .4	100 ± .8
Calcium	2.55 ± .02	2.65 ± .0	2.56 ± .05
Magnesium	1.06 ± .05	.99 ± .08	.89 ± .05

Note. Values are means ± SEs; $N = 6$.

than in other groups. This was consistent with the differences in urinary urea output.

Experiment 2

When the diet was changed from the artificial diet to *E. radiata* leaf, dry matter intake increased gradually to a level more than three times the initial intake (fig. 1*a*). However, of the urine attributes measured, only ammonium (fig. 1*b*) and glucuronic acid (fig. 1*c*) excretion mirrored the change in dry matter intake. In contrast, urine pH (fig. 1*d*) dropped rapidly and remained at a level of 5.6–5.7. Urine collected on day 1 was composed of urine stored in the bladder from the artificial diet consumed on the previous day and more acidic urine produced on the *E. radiata* diet. Collection of samples throughout the night showed that, in at least one animal, acid urine was excreted 7 h after *E. radiata* leaf was offered, although the actual time that the leaf was eaten was not observed.

This rapid decline in urine pH when *E. radiata* was introduced suggested that leaf constituents inducing acidosis were rapidly absorbed from the gut and metabolized. Although urine pH remained essentially constant, titratable

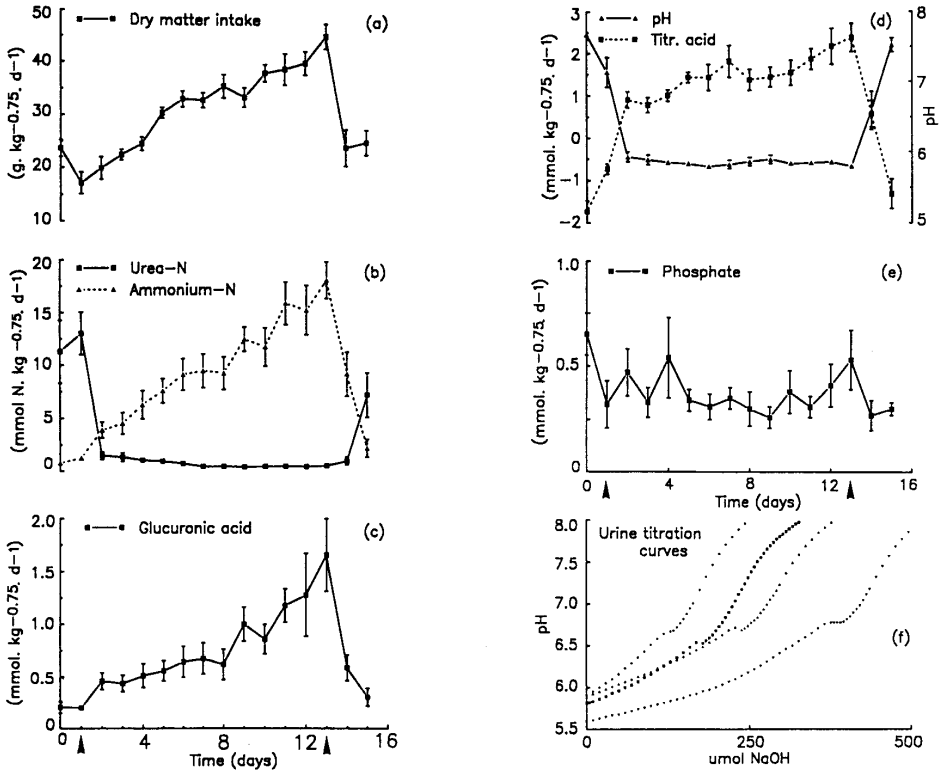

Fig. 1. Effect of changing the diet of common ringtail possums from an artificial diet to Eucalyptus radiata *foliage.* a, *Dry matter intake;* b, *urinary nitrogen excretion;* c, *urinary glucuronic acid excretion;* d, *urinary pH and titratable acid excretion;* e, *urinary phosphate excretion;* f, *four typical urine titration curves from animals eating* E. radiata *foliage. For pts.* a–e, *arrows mark the introduction and withdrawal of the* E. radiata *diet. Values are means ± SEs;* N = 6.

acid excretion more than doubled in the same period (fig. 1*d*). This suggested that the increased acid load was being well buffered. Although titration curves were consistent with phosphate's acting as the major buffer (pK' in the range 6.54–6.78) (fig. 1*f*) there was no significant relationship between titratable acid excretion and urinary phosphate (fig. 1*e*).

Experiment 3

Animals fed *E. radiata* foliage had a significantly greater nitrogen intake than those fed *E. ovata* foliage (table 5). However, an ANCOVA showed that, at similar levels of nitrogen intake, animals fed *E. radiata* foliage lost

TABLE 5
Nitrogen balance and urea kinetics in common ringtail possums fed foliage of two Eucalyptus *species*

	E. radiata	*E. ovata*
Nitrogen ($g \cdot kg^{-0.75} \cdot d^{-1}$):		
Intake	.82 ± .02	.69 ± .03
Feces	.24 ± .02	.23 ± .02
Urine	.59 ± .02	.36 ± .03
Balance	−.02 ± .02	+.10 ± .05
Urea pool ($mg\ N \cdot kg^{-0.75}$)	34.4 ± 5.9	217.4 ± 16.6
Turnover time (h)	4.98 ± .30	5.42 ± .36
Urea entry rate ($mg\ N \cdot kg^{-0.75} \cdot d^{-1}$)	168.1 ± 31.8	989.2 ± 95.5
Urea excretion rate ($mg\ N \cdot kg^{-0.75} \cdot d^{-1}$)	56.9 ± 8.5	367.4 ± 45.0
Urea degradation rate ($mg\ N \cdot kg^{-0.75} \cdot d^{-1}$)	111.3 ± 27.0	621.8 ± 80.3
Urea recycled (% of entry rate)	64.0 ± 5.8	62.3 ± 4.1

Note. Value are means ± SEs ($N = 5$).

significantly more nitrogen in the urine than did animals fed *E. ovata* foliage.

There was a higher level of urea metabolism in animals fed *E. ovata* foliage than in animals fed *E. radiata* foliage (table 5). In particular, urea pool size ($P < 0.001$) and the rate of urea formation ($P < 0.01$) were significantly higher in the animals fed *E. ovata* in spite of a lower nitrogen intake. The proportion of synthesized urea degraded in the gut was similar on both diets ($P > 0.05$), but the actual amount of urea degraded in the gut was five to six times greater ($P < 0.01$) in animals fed *E. ovata* than in animals fed *E. radiata*.

Discussion

Intake and Excretion of Nitrogen and Energy

The differences in the digestibility and metabolizability of the energy of the three diets are due largely to differences in terpene and fiber content.

The high terpene content of *Eucalyptus radiata* leaf contributed to the relatively high gross energy intake of this foliage compared to *Eucalyptus ovata.* Similarly, the relatively high digestible energy intake on this diet reflected in part the ease of absorption of terpenes in the gut (Cluff et al. 1982; White, Welch, and Flinders 1982; Foley et al. 1987). However, much of this absorbed energy was unable to be used by the body tissues and was excreted in the urine. This resulted in similar metabolizable energy intakes. The metabolizable energy intake from both foliage diets was similar to that measured by Chilcott and Hume (1984*a*) in common ringtail possums fed *Eucalyptus andrewsii* foliage and greater gliders fed *E. radiata* foliage (Foley 1987) (0.35 MJ · $kg^{-0.75}$ · d^{-1}). The higher metabolizable energy intake of animals fed the artificial diet reflected a high apparent digestibility as a consequence of its low content of fiber.

Although animals fed *E. radiata* foliage consistently excreted urine composed primarily of ammonium nitrogen rather than urea nitrogen, there was no consistent effect on total urinary nitrogen excretion. Studies in humans and rats suggest that, where nitrogen is not limiting, total urinary nitrogen excretion remains constant, even though the ratio of urinary urea to ammonium changes dramatically (Oliver and Bourke 1975; Fine, Carlyle, and Bourke 1977). However, when the supply of nitrogen is scarce, lean body mass may be catabolized to provide glutamine for ammoniagenesis (Hannaford et al. 1982). In experiment 3, an ANCOVA showed that animals fed *E. radiata* foliage lost significantly more nitrogen in the urine than did those fed *E. ovata* foliage. Since all animals fed on leaf from one tree in this experiment, the results may be the most reliable. Further, studies on the urea metabolism of these animals showed that acidotic animals recycled only a small amount of nitrogen back to the gut. Therefore, the finding that urinary nitrogen was increased was not unexpected. Further studies of acidotic animals fed artificial diets will be required to resolve this important point.

Relation of Urinary Acidification to Detoxification

Absorbed terpenes and phenolics are detoxified by a two-stage process consisting of the structural modification of the molecule, often by mixed-function oxidases in the liver, followed by conjugation with a small molecule such as glucuronic acid, sulfate, or glycine (Caldwell 1982). The detoxification process is broadly similar in all mammalian herbivores. In arboreal marsupials, conjugation with glucuronic acid predominates (Hinks and Bolliger 1957; Baudinette et al. 1980). Irrespective of the compound involved, the effect of conjugation is to convert substances that are lipophilic into

more polar, hydrophilic compounds that can be excreted in the urine or bile. However, this also has the effect of converting weakly acidic or neutral compounds into strong organic acids. For example, the dissociation constants of a range of conjugated compounds (including glucuronide conjugates of terpenes) reported by Robinson, Smith, and Williams (1953) lay between three and four.

The elevation of urinary titratable acidity and ammonium and the decline in urinary urea and pH when animals fed on *E. radiata* are indicative of a severe metabolic acidosis. Although there was a complex mixture of organic acids excreted in the urine (many of which remain unidentified [A. Duffield and W. J. Foley, unpublished data]), the finding that glucuronic acid was the major part of the titratable acid excretion supports the hypothesis that acidosis results from the accumulation of organic acids produced during the detoxification of dietary terpenes and phenolics.

The classical view is that urinary ammonium represents the net excretion of proton from the body. Pitts (1964) concluded that H^+ generated from carbonic acid in renal tubular cells combined in acid urine with ammonia derived from glutamine and was eliminated from the body as NH_4^+. However, the product of glutamine catabolism is not ammonia (NH_3), but ammonium (NH_4^+) (Atkinson and Bourke 1987), and so the excretion of this ammonium ion in the urine cannot represent the net excretion of protons from the body. Since urea production consumes bicarbonate, the net effect of ammonium excretion is to conserve bicarbonate to titrate excess acid—in this case metabolically produced organic acid.

Renal Acidification

Although examination of the urine provided strong evidence that the animals fed *E. radiata* foliage were acidotic, this was not reflected in the blood. In particular, blood pH was similar on all treatments, and, although concentrations of bicarbonate were lower in the plasma of animals fed *E. radiata* than *E. ovata,* they were similar to those of animals fed the artificial diet. However, the exact relationship between systemic pH and renal acidification is uncertain. In particular, Schwartz and Cohen (1978) have summarized a large amount of evidence that suggests that the mechanisms of acid excretion are linked with the sodium supply to the distal nephron and largely independent of changes in systemic pH. This hypothesis rests partly on experiments of De Sousa et al. (1974), who found no evidence of systemic acidosis in dogs fed nitric acid, whereas hydrochloric acid had a marked acidotic effect.

However, a recent reexamination of these experiments by Madias and Zelman (1986) showed that transitory acidosis does occur with all acid treatments but that the timing of De Sousa et al.'s (1974) measurements were such that these effects were missed. In spite of this, it is still true that the reabsorbability of the accompanying acid anion mediates the severity of the acidosis induced. Poorly reabsorbed anions such as nitrate (Møller and Sheikh 1983) will be delivered rapidly with endogenous sodium to the distal nephron, whereas anions such as chloride will be largely reabsorbed and reenter the circulation. For example, sodium-depleted subjects excreted acid urine when infused with sodium sulfate, but sodium chloride had little effect on urine acidity (Schwartz and Cohen 1978).

Anions of conjugated compounds such as glucuronides and hippurates are only poorly reabsorbed by the kidney tubules (Møller and Sheik 1983). Therefore, they should be rapidly excreted, and any systemic acidosis due to their formation should be only transitory. The rapid absorption of *Eucalyptus* terpenes together with the formation of an acid urine within a maximum of 7 h of commencing feeding on *E. radiata* leaf suggests that this is indeed the case. Future studies need to characterize plasma acid-base status several times during and after feeding.

Effects of Acidosis on Urea Metabolism

The low concentration of urinary urea in animals fed *E. radiata* foliage reflected a lower rate of urea synthesis rather than a greater urea recycling rate. Therefore, even although animals on both diets recycled similar proportions of the daily urea production, the quantitative importance of urea degradation in the gut was much greater in animals fed *E. ovata* diets than in those fed *E. radiata* diets. Chilcott and Hume (1984*b*) have argued that urea recycled to the hindgut of common ringtail possums is an important source of nitrogen to be incorporated into microbial protein and later ingested as caecotrophs. Clearly, acidosis has a major impact on this route of nitrogen recycling by influencing the partitioning of urinary nitrogen between urea and ammonium. If animals rely on recycled urea to help meet nitrogen requirements, as suggested by Chilcott and Hume (1984*b*), then their acid-base status will be an important determinant of nitrogen balance.

The level of urea metabolism in common ringtail possums in Chilcott and Hume's (1984*b*) experiments was even lower than those recorded here. The animals were fed *E. andrewsii* foliage, and urea pool size was 30% and urea entry rates 25% of the present values for *E. radiata* foliage. Similar low levels of urea metabolism were reported in greater gliders fed *E. radiata* foliage and in common brushtail possums fed *Eucalyptus melliodora* foliage.

Some of the differences can be attributed to variations in nitrogen intake, but it is likely that there is a variable acidosis in all three animals.

The importance of urea in the formation of concentrated urine in at least some species (Jamison 1981) suggests that chronically low levels of urea synthesis may also have an impact on the water requirements of the animals as well as their nitrogen requirements. This possibility should receive further study.

Possible Role of Acid Excretion in Limiting Food Intake

The maintenance of pH homeostasis is the most important regulatory necessity of any mammal. Therefore, processes that threaten acid-base balance are likely to be closely controlled. Different species of *Eucalyptus* leaf give rise to different acid loads, the absorption and metabolism of acid-inducing components of the diet is very rapid, and acidosis can have a major impact on the ability of animals to conserve nutrients. I suggest that these effects are the consequences of the detoxification of plant allelochemicals. However, irrespective of the cause of the acid loads, animals should be able to consume "acid-producing" leaf species only at a rate that does not exceed their ability to buffer and excrete the acid loads produced. Alternatively, they must choose leaf species that minimize the additional acid loads produced. Changes in intracellular pH are the most likely signal that tells an animal to slow or cease feeding on particular diet items. The observation that acid urines can be formed and excreted within 7 h of the animal's commencing to feed suggests that responses to acidosis are sufficiently rapid to affect feeding in the same night. The ability of animals to buffer diet-induced acid loads would therefore appear to be important.

Urine titration curves suggested that phosphate was the major buffer of the urine of common ringtail possums, since the pK′ values observed (fig. 1*f*) were within the range observed in studies of other species and close to the theoretical value (6.8) (Schwartz, Bank, and Cutler 1959). This was expected, since phosphate is well recognized as the major buffer of mammalian urine (Hamm and Simon 1987). It was therefore surprising that changes in urinary phosphate excretion did not reflect either the pattern of food intake or titratable acid excretion.

Australian forests are notoriously low in phosphorus, and arboreal mammals are concentrated in areas where foliar phosphorus concentrations are highest (Braithwaite, Dudzinski, and Turner 1983). Chronic metabolic acidosis results in an increased urinary phosphate excretion (Hamm and Simon 1987), and this may increase the problems of obtaining sufficient phosphorus for animals. Clearly, studies of other animal species are needed to assess

the importance of phosphate in acid excretion and also to determine whether acidotic effects are peculiar to those animals that eat *Eucalyptus* foliage or a general response to diets rich in absorbable allelochemicals.

Acknowledgments

I wish to thank Kodak (Australasia) Proprietary Limited for allowing use of an Ektachem clinical chemistry analyzer, Mr. George Streitberg for assistance with blood gas measurements, Dr. Ian McDonald for help sampling blood, and Ms. Jillian Sass for excellent technical assistance. The study was supported by a Monash University Research Fellowship, and I thank Dr. Gordon Sanson for facilitating this and for his comments on the manuscript.

Literature Cited

ATKINSON, D. E., and E. BOURKE. 1987. Metabolic aspects of the regulation of systemic pH. Am. J. Physiol. 252:F947–F956.

BAUDINETTE, R. V., J. F. WHELDRAKE, S. HEWITT, and D. HAWKE. 1980. The metabolism of [^{14}C] phenol by native Australian rodents and marsupials. Aust. J. Zool. 28:511–520.

BLUMENKRANTZ, N., and G. ASBOE-HANSEN. 1973. New method for the quantitative determination of uronic acids. Anal. Biochem. 54:484–489.

BRAITHWAITE, L. W., M. L. DUDZINSKI, and J. TURNER. 1983. Studies on the arboreal marsupial fauna of eucalypt forests being harvested for woodpulp at Eden, N.S.W. II. Relationship between the fauna density, richness and diversity and measured variables of the habitat. Aust. Wildl. Res. 10:231–247.

CALDWELL, J. 1982. The conjugation reactions in foreign compound metabolism: definition, consequences and species variation. Drug Metab. Rev. 13:745–778.

CHILCOTT, M. J., and I. D. HUME. 1984*a*. Digestion of *Eucalyptus andrewsii* foliage by the common ringtail possum, *Pseudocheirus peregrinus*. Aust. J. Zool. 32:605–613.

———. 1984*b*. Nitrogen and urea metabolism and nitrogen requirements of the common ringtail possum (*Pseudocheirus peregrinus*) fed *Eucalyptus andrewsii* foliage. Aust. J. Zool. 32:615–622.

CLUFF, L. K., B. L. WELCH, J. C. PEDERSON, and J. D. BROTHERSON. 1982. Concentration of monoterpenoids in rumen ingesta of wild mule deer. J. Range Manage. 35:192–194.

COCHRAN, W. G., and G. M. COX. 1957. Experimental designs. 2d ed. Wiley, New York.

COCIMANO, M. R., and R. A. LENG. 1967. Metabolism of urea in sheep. Br. J. Nutr. 21: 353–371.

CORK, S. J., I. D. HUME, and T. J. DAWSON. 1983. Digestion and metabolism of a natural foliar diet (*Eucalyptus punctata*) by an arboreal marsupial, the koala (*Phascolarctos cinereus*). J. Comp. Physiol. 153B:181–190.

CORK, S. J., and L. PAHL. 1984. The possible influence of nutritional factors on diet and habitat selection by the ringtail possum. Pages 269–276 in A. P. SMITH and I. D. HUME, eds. Possums and gliders. Australian Mammal Society, Sydney.

CROCKER, C. L. 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinization. Am. J. Med. Technol. 33:361–365.

DE SOUSA, R. T., J. C. HARRINGTON, E. S. RICANATI, J. W. SHELKROT, and W. B. SCHWARTZ. 1974. Renal regulation of acid-base equilibrium during chronic administration of mineral acid. J. Clin. Invest. 53:465–476.

EBERHARD, I. H., J. MACNAMARA, R. J. PEARSE, and I. A. SOUTHWELL. 1975. Ingestion and excretion of *Eucalyptus punctata* D.C. and its essential oil by the koala, *Phascolarctos cinereus* (Goldfuss). Aust. J. Zool. 23:169–179.

FINE, A., J. E. CARLYLE, and E. BOURKE. 1977. The effects of administrations of HCl, NH_4Cl and NH_4HCO_3 on the excretion of urea and ammonium in man. Eur. J. Clin. Invest. 7:587–589.

FOLEY, W. J. 1987. Digestion and energy metabolism in a small arboreal marsupial, the greater glider (*Petauroides volans*), fed high-terpene *Eucalyptus* foliage. J. Comp. Physiol. 157B:355–362.

FOLEY, W. J., and I. D. HUME. 1987. Nitrogen requirements and urea metabolism in two arboreal marsupials, the greater glider (*Petauroides volans*) and the brushtail possum (*Trichosurus vulpecula*), fed *Eucalyptus* foliage. Physiol. Zool. 60:241–250.

FOLEY, W. J., E. V. LASSAK, and J. BROPHY. 1987. Digestion and absorption of *Eucalyptus* essential oils in greater glider (*Petauroides volans*) and brushtail possum (*Trichosurus vulpecula*). J. Chem. Ecol. 13:2115–2130.

FOX, L. R., and B. J. MACCAULEY. 1977. Insect grazing on *Eucalyptus* in response to variation in leaf tannins and nitrogen. Oecologia 29:145–162.

GOERING, H. K., and P. J. VAN SOEST. 1970. Forage fiber analyses. Agric. Handbook 379. U.S. Department of Agriculture, Washington, D.C.

HAMM, L. L., and E. E. SIMON. 1987. Roles and mechanisms of urinary buffer excretion. Am. J. Physiol. 253:F595–F605.

HANNAFORD, M. C., M. G. GOLDSTEIN, R. G. JOSSE, and M. L. HALPERIN. 1982. Role of acidosis in the protein wasting of fasting in the rat and the rabbit. Can. J. Physiol. Pharmacol. 60:331–334.

HEISLER, N. G. 1989. Parameters and methods in acid-base physiology. Pages 305–332 in C. R. BRIDGES and P. J. BUTLER, eds. Techniques in comparative respiratory physiology. Cambridge University Press, Cambridge.

HINKS, N. T., and A. BOLLIGER. 1957. Glucuronuria in a herbivorous marsupial, *Trichosurus vulpecula*. Aust. J. Exp. Biol. Med. Sci. 35:37–46.

JAMISON, R. L. 1981. Urine concentration and dilution: The roles of antidiuretic hormone and urea. Pages 495–550 in B. BRENNER and F. C. RECTOR, eds. The kidney. Saunders, Philadelphia.

MADIAS, N. E., and S. J. ZELMAN. 1986. The renal response to chronic mineral acid feeding: a re-examination of the role of systemic pH. Kidney Int. 29:667–674.

MARKS, D. L., T. SWAIN, S. GOLDSTEIN, A. RICHARDS, and M. LEIGHTON. 1988. Chemical correlates of rhesus monkey food choice: the influence of hydrolysable tannins. J. Chem. Ecol. 14:213–235.

MØLLER, J. V., and M. I. SHEIKH. 1983. Renal organic anion transport system: pharmacological, physiological and biochemical aspects. Pharmacol. Rev. 34:315–358.

MORROW, P. A., and L. R. FOX. 1980. Effects of variation in *Eucalyptus* essential oil yield on insect growth and grazing. Oecologia 45:209–219.

OATES, J. F., P. G. WATERMAN, and G. M. CHOO. 1980. Food selection by the south Indian leaf monkey *Presbytis johnii* in relation to leaf chemistry. Oecologia 45: 45–56.

OLIVER, J., and E. BOURKE. 1975. Adaptations in urea ammonium excretion in metabolic acidosis in the rat: a reinterpretation. Clin. Sci. Mol. Med. 48:515–520.

PITTS, R. F. 1964. Renal production and excretion of ammonia. Am. J. Med. 36:720–742.

ROBBINS, C. T., S. MOLE, A. E. HAGERMAN, and T. A. HANLEY. 1987. Role of tannins in defending plants against ruminants: reduction in dry matter digestion? Ecology 68:1606–1615.

ROBINSON, D., J. N. SMITH, and R. T. WILLIAMS. 1953. Studies in detoxication. LII. The apparent dissociation constants of some glucuronides, mercapturic acids and related compounds. Biochem. J. 55:151–155.

SCHWARTZ, W. B., N. BANK, and R. W. P. CUTLER. 1959. The influence of urinary ionic strength on phosphate pK'_2 and the determination of titratable acid. J. Clin. Invest. 38:347–355.

SCHWARTZ, W. B., and J. J. COHEN. 1978. The nature of the renal response to chronic disorders of acid-base equilibrium. Am. J. Med. 64:417–428.

SIMMONS, D., and R. F. PARSONS. 1987. Seasonal variation in the volatile leaf oils of two *Eucalyptus* species. Biochem. Syst. Ecol. 15:209–215.

SOUTHWELL, I. A. 1978. Essential oil content of koala food trees. Pages 62–74 in T. J. BERGIN, ed. The koala. Zoological Parks Board of New South Wales, Sydney.

WHITE, S. M., B. L. WELCH, and J. T. FLINDERS. 1982. Monoterpenoid content of pygmy rabbit stomach ingesta. J. Range Manage. 35:107–109.

WRONG, O., and H. E. F. DAVIES. 1959. The excretion of acid in renal disease. Q. J. Med., N.S., 28:259–314.