Chapter 4

DIGESTION OF EUCALYPTUS FOLIAGE BY THE GREATER GLIDER AND BRUSHTAIL POSSUM

4.1 Introduction

The evaluation of the nutritive value of *Eucalyptus* foliage to arboreal marsupials depends upon measurement of its chemical composition and the proportion of the various foliage components that are digested. While *Eucalyptus punctata* foliage dry matter was found to be digested to the extent of 54% by Koalas (Cork *et al.*, 1983), smaller folivores such as the Greater Glider and Brushtail Possum could be expected to have difficulties meeting their energy requirements from diets high in structural carbohydrates (Parra, 1978; Van Soest, 1982).

Little is known of the intake and digestion of *Eucalyptus* foliage by Greater Gliders. Marples (1973) estimated dry matter intake by free-living Greater Gliders to be only 20 g per day, but this would seem to be insufficient to satisfy even their basal metabolism requirements (Section 1.2). While Wellard and Hume (1981b) have shown that Brushtail Possums are able to digest 55-60% of the cell walls of semi-purified diets, nothing is known of the ability of this species to digest eucalypt foliage cell walls. In the studies described in this chapter, measurements have been made of the chemical composition of both *E. radiata* and *E. melliodora* leaves at different times of the year and from different parts of the trees. The digestion of dry matter, cell contents and several components of the cell walls of *E. radiata* (Greater Glider) and *E. melliodora* (Brushtail Possum) was investigated by a series of feeding experiments and subsequent chemical analysis of the diet and excreta.

In several recent studies of digestion in domestic herbivores (Akin *et al.*, 1973, 1974, Harbers *et al.*, 1981) traditional determinations of digestibility have been supported by observations of the remnants of the plant tissues by transmission and scanning electron microscopy. These

techniques have provided information on the structural characteristics of the plant tissues that influence attachment of micro-organisms and subsequent digestion (Akin, 1979, Akin and Barton, 1983). Therefore, in addition, observations were made with a scanning electron microscope of digesta and faecal remnants to complement the results obtained from the more traditional approach.

P A R T A

COMPOSITION OF THE DIET

4.2. Materials and Methods

Samples of the leaves offered to each species in the feeding experiments were collected as described previously (Section 2.2.3). They were analysed for dry matter, nitrogen, gross energy, phenolics and fibre components in every case and for crude lipid, total non-structural carbohydrate and essential oils occasionally. These techniques are outlined in Section 2.4. In autumn, 1981, samples of shoots, mature leaves and old leaves were collected from a single *E. radiata* and *E. melliodora* tree and immediately frozen in liquid nitrogen. These were analysed for dry matter, organic matter, total nitrogen, total non-structural carbohydrate, crude lipid, gross energy and cell wall components as described in Section 2.4.

The proportion of total nitrogen occurring as non-protein nitrogen was assessed by macerating leaves from four *E. radiata* and four *E. melliodora* trees in a borate buffer (0.2M pH 7.6; King, 1971) and determining the nitrogen content of the supernatant before and after the addition of 10% TCA.

Several tests were made of the effect of the inclusion of Na_2SO_3 and of sequential detergent extractions on the determination of fibre

TABLE 4.1a: *Composition of E. radiata foliage eaten by Greater Gliders during different experiments (all values except moisture and energy as % DM)*

Constituent	P1	P2	P3	P4[1]	P5[1]	P6	P7	P8
Moisture (% wet weight)	57.9±0.4	57.0±1.0	57.7±0.7	54.6±0.6	56.1±0.4	54.3±0.4	57.8±0.9	64.5±0.6
Organic matter	97.7±0.3	96.9±0.2	--	--	--	--	98.9±0.2	98.3±0.3
Total nitrogen	1.63±0.02	1.56±0.01	2.02±0.01	2.02	1.97	1.90	2.19	1.90
Total non-structural carbohydrate	8.8±0.3	7.6±0.1	--	--	--	--	--	--
Crude lipid	14.6±0.5	13.9±0.7	--	--	--	--	--	--
Essential oil w/v	--	--	--	--	8.8	--	--	13.2
Total phenols	21.8±1.6	14.3±0.7	22.7±0.9	23.5	18.4	18.6	9.2±0.8	11.1±0.7
CELL WALLS								
NDF	35.8±0.1	40.0±0.1	32.2±0.0	30.4	31.6	32.6	30.0±0.0	34.5±0.4
"Hemicellulose"[2]	2.9±0.0	-1.0±0.2	4.0±0.0	2.9	1.9	0.3	2.0±0.1	2.1±0.1
ADF	32.9±0.1	40.9±0.1	28.1±0.0	27.5	29.7	32.3	28.0±0.1	32.5±0.5
"Cellulose"[3]	17.0±0.1	19.1±0.1	14.6±0.1	13.5	15.5	13.6	13.3±0.2	12.6±0.4
Lignin	16.0±0.2	21.8±0.1	13.6±0.2	14.0	14.2	18.7	14.7±0.2	19.9±0.4
Residual Ash	0.1±0.1	0.3±0.0	0.3±0.0	0.4	0.1	0.0	0.3±0.2	0.1±0.1
Gross energy ($kJ.g^{-1}$)	23.7±0.1	22.3±0.0	23.7±0.1	23.5	24.0	24.6	24.0±0.1	24.5±0.1

[1] Analyses made on single bulked sample; [2] NDF-ADF; [3] ADF-Lignin

TABLE 4.1b: *Composition of E. melliodora foliage eaten by Brushtail Possums during different experiments (all values except moisture and energy as % DM)*

Constituent	T1	T2	T3	T4[1]	T5[1]	PEG1	PEG2
Moisture (% wet weight)	49.4±0.1	50.1±0.2	50.0±0.1	49.8±0.3	50.2±0.3	50.9±0.6	51.7±0.4
Organic matter	96.2±0.2	96.1±0.3	--	--	--	--	97.0±0.2
Total nitrogen	1.58±0.04	1.67±0.2	1.62±0.03	1.65±0.04	1.67±0.4	1.78±0.02	1.43±0.01
Total non-structural carbohydrate	8.9±0.6	--	10.2±0.3	--	--	--	--
Crude lipid	10.9±0.4	--	11.0±0.2	--	--	--	--
Essential oil	0.9[1]	--	--	--	--	--	1.2[1]
Total phenols	27.9±0.3	29.1±1.2	27.9±1.7	27.0	27.1	24.8±0.5	19.9±0.9
CELL WALLS							
NDF	29.6±0.4	26.6±0.6	27.7±0.4	28.2	28.8	30.3±0.1	29.5±0.3
"Hemicellulose"	5.3±0.6	4.8±0.4	4.2±0.5	4.8	5.8	4.5±0.4	6.2±0.4
ADF	24.3±0.8	21.8±0.5	23.5±0.2	23.4	23.0	25.9±0.3	23.3±0.2
"Cellulose"	14.2±0.6	12.8±0.3	14.7±0.2	14.7	13.0	16.1±0.5	11.1±0.3
Lignin	10.1±0.2	9.0±0.7	8.7±0.2	8.7	10.0	9.8±0.3	12.2±0.3
Residual Ash	0.1±0.0	0.1±0.0	0.1±0.0	0.2	0.1	0.1±0.0	0.4±0.0
Gross energy ($kJ.g^{-1}$)	20.6±0.1	20.6±0.2	20.9±0.1	21.5	21.1	21.0±0.0	21.1±0.2

[1] Single bulked sample

TABLE 4.2: *Variation in foliage constituents within a single E. radiata and a single E. melliodora tree (values except moisture and energy as % DM)*

Constituent	*E. radiata*			*E. melliodora*		
	Shoots	Mature leaves	Old leaves	Shoots	Mature leaves	Old leaves
Moisture (% wet weight)	65.1	57.4	51.2	62.0	50.7	46.9
Organic matter	98.8	98.8	98.6	98.2	97.1	97.1
Total nitrogen	1.83	1.76	1.57	1.63	1.38	1.27
Total non-structural carbohydrate	10.6	8.8	8.0	11.6	10.8	9.2
Crude lipid	14.8	15.2	13.7	8.3	9.2	10.1
Essential oil (v/w)	11.9	9.8	--	1.1	0.8	--
CELL WALLS						
NDF	19.8	29.2	32.3	17.7	25.5	29.1
"Hemicellulose"[1]	1.1	5.5	5.0	4.1	1.5	1.7
ADF	18.7	23.7	27.3	13.6	24.0	27.4
"Cellulose"[2]	10.0	14.5	14.3	7.6	12.9	12.7
Lignin	8.7	9.2	13.0	6.0	11.1	14.7
Residual ash	0.1	0.2	0.5	0.1	0.1	0.1
Gross energy ($kJ.g^{-1}$)	21.6	21.9	22.1	19.9	20.1	20.3

[1] NDF-ADF; [2] ADF-Lignin

components in diet and faeces samples from both species. Acetyl bromide-soluble lignin was extracted from diet and faecal samples of the two species by the methods of Morrison (1972a,b). The UV spectra of these extracts were recorded on a Pye Unicam UV spectrophotmeter.

4.3 **Results**

The chemical composition of *E. radiata* and *E. melliodora* foliage consumed by Greater Gliders and Brushtail Possums during feeding experiments is shown in Tables 4.1a and 4.1b. More detailed analyses of the phenolic compounds and essential oils are given in Chapters 6 and 7 respectively. Few seasonal trends were apparent although the moisture content of leaves used in the main summer experiment with the Greater Gliders was the highest encountered in the study. Between 89 and 94 % of the dry matter of *E. radiata* and 90-91% of the dry matter of *E. melliodora* could be accounted for in the measured components. Total phenolics were the major soluble component in both species, followed by crude lipid. Both species were highly lignified, the ratio of lignin to ADF being 0.5 in *E. radiata* and 0.4 in *E. melliodora*.

Table 4.2 shows the variation in the chemical composition of different leaf age classes within individual trees. Older leaves generally had lower contents of moisture, nitrogen and total non-structural carbohydrates but higher contents of cell wall constituents and, in particular, lignin. However, the ratio of lignin to ADF was relatively constant throughout leaf age classes. This trend held for both species.

The addition of Na_2SO_3 to neutral-detergent extractions (Table 4.3 a,b) led to a decrease in recovered residue of 3-4 percentage units in *E. melliodora* leaf and Brushtail Possum faeces but 6-7 percentage units in *E. radiata* leaf and Greater Glider faeces. Separate sample ADF values were similarly higher than sequentially determined ADF (with sulphite), but omitting sulphite from the pre-extraction halved these differences. In faecal samples, these differences could be mostly accounted for by corresponding differences in the "lignin" fraction, but both "cellulose"

TABLE 4.3a: *Effect of sequential extractions and sodium sulphite on the determination of cell wall constituents of E. radiata and Greater Glider faeces*

Object	Subject					
	Whole leaf or faeces	NDR[1] +S[2]	NDR -S[3]	ADR[4] whole leaf/ faeces	ADR of NDR+S	ADR of NDR-S
E. radiata						
NDF+S	34.11					
NDF-S	40.98					
ADF	30.34	24.97	27.13			
Cellulose				13.37	9.53	8.77
Lignin				16.97	15.44	18.36
Faeces						
NDF+S	59.65					
NDF-S	65.89					
ADF	53.84	45.44	51.30			
Cellulose				16.73	16.10	17.52
Lignin				37.11	29.34	33.78

[1] Neutral detergent residue

[2] With Na_2SO_3

[3] Without Na_2SO_3

[4] Acid detergent residue

TABLE 4.3b: *Effect of sequential extractions and sodium sulphite on the determination of the cell wall constituents of E. melliodora and Brushtail Possum faeces*

Object	Subject					
	Whole leaf or faeces	NDR +S	NDR -S	ADR whole leaf/ faeces	ADR of NDR+S	ADR of NDR-S
E. melliodora						
NDF+S	30.33					
NDF-S	33.79					
ADF	23.50	19.04	20.32			
Cellulose				11.55	10.04	9.22
Lignin				11.95	9.00	11.10
Faeces						
NDF+S	43.87					
NDF-S	47.30					
ADF	36.85	30.81	32.37			
Cellulose				14.26	15.11	14.41
Lignin				22.59	15.70	17.96

Abbreviations as per Tabe 4.3a

and "lignin" fractions of leaves were affected by NDF and sulphite pretreatments.

The UV spectra of the acetyl bromide-soluble lignin of feed and faeces samples are shown in Figure 4.1 Faecal lignins differed markedly from the corresponding feed lignins, especially in the region 300-340nm. The major differences in the leaf samples was between 250-280nm where *E. radiata* was characterized by a large peak absent or reduced in *E. melliodora*.

In both species only 5-10% of the total leaf nitrogen could be extracted with the borate buffer. Of this, between 50 and 100% could be precipitated by TCA. However, the small amount of nitrogen in the supernatants limited the accuracy of the nitrogen determinations.

4.4 Discussion

Between 89 and 94% of the dry matter of *E.radiata* and 90-91% of *E. melliodora* could be accounted for as crude protein, crude lipid, nonstructural carbohydrate, phenolics, cell wall constituents and ash. Essential oils of *E. radiata* (9-13% DM) could account for the most of the remaining fraction in this species but oils were at most only 1-1.5% of *E. melliodora* dry matter. Nonetheless, although most of the dry matter can be accounted for, there are probably some components that occurred in the leaves that are not included in any of the above categories. Pectins are water-soluble carbohydrates associated with the cell wall that are usually extracted by neutral-detergent solutions (Bailey and Ulyatt, 1970, Bailey *et al.*, 1978). There have been no measurements made of the pectin content of any eucalypt species. Mould and Robbins (1981) found pectin contents of between 6 and 11% in leaves from a range of North American trees browsed by Elk, which is similar to typical values found in grasses (Bailey and Ulyatt, 1970). Additionally, while some surface leaf waxes would have been included in the crude lipid fraction, other wax compounds are insufficiently polar to have been extracted by the solvents used here (Horn *et al.*, 1964).

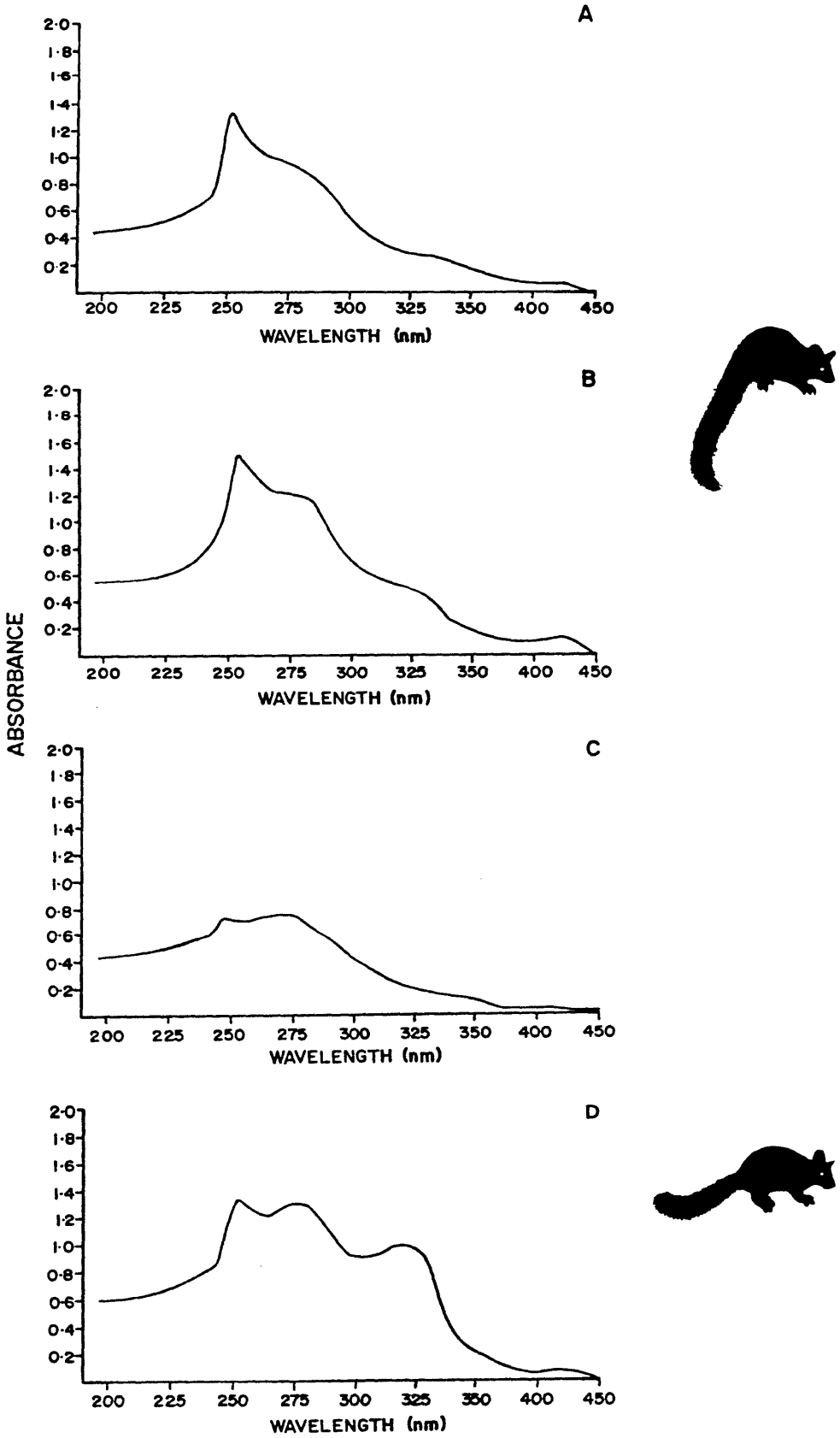


FIGURE 4.1: *U-V spectra of acetyl bromide-soluble lignin from (A)* E. radiata, *(B) Greater Glider faeces, (C)* E. melliodora, *(D) Brushtail Possum faeces.*

There may also be some overlap between some components of the above categories. For example, attempts to further partition the crude lipid fraction into component long chain fatty acids were prevented by contamination by essential oils in both species but in particular *E. radiata*. Secondly, the data above suggest that not all the leaf nitrogen occurs as protein nitrogen and that 6.25 is not necessarily the most appropriate factor for converting nitrogen to crude protein in eucalypt leaves. In a range of tropical leaf samples Milton and Dintzis (1981) found that a factor of 4.5 provided a more accurate conversion of total nitrogen to crude protein nitrogen. The balance of the nitrogen in eucalypts is most likely free amino acids but may also include acid amides, nitrogenous fats or ammonium salts (Milton and Dintzis, 1981). Journet and Cochrane (1978) found that up to 50% of the nitrogen of *E. blakelyi* leaves occurred as free amino acids.

Although the detergent system is recognized as the best means of partitioning plant cell wall constituents into nutritionally meaningful entities (Van Soest, 1982), several problems exist with the procedures. Ideally, the neutral-detergent residue contains hemicellulose, cellulose, lignin and residual ash, while extraction with acid detergent should quantitatively remove the hemicellulose. The use of Na_2SO_3 in the NDF extraction has been criticized since it has been shown that sulphite can solubilize some hemicellulose (Mould and Robbins, 1981) as well as some lignin and cutin (Hartley, 1972). Na_2SO_3 is included in the extraction to ensure the complete removal of protein and the higher retention of lignin and hemicellulose in the neutral-detergent residue (without sulphite) (Table 4.3a,b) is probably offset by an increased protein content. Solubilization of hemicellulose may partly explain why in the present study separate-sample ADF was higher than sequentially-determined ADF. Other authors have shown contamination of separate-sample ADF by hemicellulosic sugars (Morrison, 1980, Theander and Aman, 1980). Since hemicellulose and cellulose are determined by difference, uncertainties in the composition of ADF and NDF may lead to errors in the determination of these other components. The 72% H_2SO_4 lignin fraction may also include Maillard polymers, plant cuticle and tannin complexes. Maillard polymers may be formed by non-enzymic browning reactions if samples are dried at

TABLE 4.4: *Composition of the mature foliage of diets offered to or selected by some arboreal folivores (values expressed as percentage dry matter)*

Species	Diet	Water	Nitrogen	NDF	ADF	Lignin	Total phenolics	Lipid	TNC	Ash	Reference
EUTHERIANS											
Howler Monkey	Fruit, young and mature foliage	-	12	39	-	-	6-11	-	1-7	-	Milton (1979) Milton *et al.* (1980)
Black Colobus	Seeds and foliage	-	12	-	58	-	7	-	4	5	McKey *et al.* (1981)
Leaf Monkey	Young and mature foliage	72	15	33	24	12	4	-	-	-	Oates *et al.* (1980)
MARSUPIALS											
Koala	*E. punctata*	52	8	34	29	14	25	17	11	3	Cork (1984)
	Mixed eucalypt leaf	62-66	11-13	24-27	19-23	5-8	-	9-11	-	4	Ullrey *et al.* (1981b)
Ringtail Possum	*E. andrewsii*	59	7	51	42	20	-	-	-	-	Chilcott (1982)
	Eucalyptus and *Rubus*	-	6-12	48-51	23-43	8-27	12-17	-	-	-	Cork and Pahl (1984)
Greater Glider	*E. radiata*	58	12	33	32	17	18	14	8	2	Present study
Brushtail Possum	*E. melliodora*	51	10	29	24	10	26	11	10	4	Present study
	Pseudopanax arboreum	-	7	20	17	10		-	17	6	Fitzgerald (1978)

TABLE 4.5: *Body weight and body weight change of Greater Gliders and Brushtail Possums during feeding experiments (Mean ± SE)*

Species	Experiment	Season	Mean body weight (kg)	Mean body weight change (kg)
Greater Glider	P1	Summer 1981	1.11 ± 0.04	+0.01 ± 0.01
	P2	Autumn 1981	1.12 ± 0.04	0.00 ± 0.03
	P3	Autumn 1981	1.15 ± 0.03	+0.01 ± 0.01
	P4	Autumn 1981	1.15 ± 0.03	-0.01 ± 0.01
	P5	Winter 1981	1.12 ± 0.03	-0.01 ± 0.00
	P6	Winter 1981	1.14 ± 0.03	+0.02 ± 0.01
	P7	Spring 1981	1.12 ± 0.04	+0.02 ± 0.01
	P8	Summer 1981	1.14 ± 0.03	-0.03 ± 0.01
Brushtail Possum	T1	Winter 1981	2.38 ± 0.10	0.00 ± 0.01
	T2	Spring 1981	2.33 ± 0.16	0.00 ± 0.01
	T3	Summer 1982	2.59 ± 0.23	+0.04 ± 0.02
	T4	Summer 1982	2.55 ± 0.27	+0.04 ± 0.11
	T5	Summer 1982	2.59 ± 0.25	+0.01 ± 0.00
	PEG1 (1)	Autumn 1982	2.65 ± 0.24	+0.04 ± 0.03
	PEG1 (2)	Autumn 1982	2.68 ± 0.24	0.00 ± 0.03
	PEG2 (1)	Autumn 1982	2.51 ± 0.17	+0.02 ± 0.01
	PEG2 (2)	Autumn 1982	2.54 ± 0.15	-0.02 ± 0.03

high temperatures (Van Soest, 1982). This problem was avoided by freeze-drying the samples or by oven-drying at only 55° C.

Clearly, a detailed study of the composition of the detergent residues of eucalypt leaf and the mode of action of potential interfering substances is required. Although such an investigation was outside the scope of the present work, the use of consistent procedures over a wide number of samples gives confidence that the results obtained are realistic estimates of the utilization of the fibre fraction by the two possum species.

Tables 1.1 and 4.4 give details of the composition of some other herbivore diets. Eucalypt leaves are relatively low in protein and some potential structural inhibitors such as silica. On the other hand, although the levels of cell wall constituents are at the low end of the range of values found in mature leaves, this fibre is highly lignified. Leaves (and fruits) consumed by several primate arboreal folivores (eg. Lar Gibbon and Leaf Monkey) are similarly highly lignified. The next section will consider some of the effects of a high degree of lignification on the digestibility of eucalypt foliage.

P A R T B

DIGESTION OF FOLIAGE COMPONENTS

4.5 **Materials and Methods**

(a) **Animals:** A total of 13 adult Greater Gliders was used in the feeding experiments (Table 4.5). However, a core group of five animals (four females, one male) was common to all experiments except Experiment P7, a slaughter experiment in connection with the experiments described in Chapter 9. Seven adult male Brushtail Possums were used in the five feeding experiments, although a core group of three animals ate the foliage diets most consistently.

TABLE 4.6a: *Intake, excretion and apparent digestibility of dry matter by Greater Gliders (values expressed as* $g.kgW^{-0.75}.d^{-1}$*) (Mean ± SE)*

Experiment	Intake	Faecal excretion	Digestibility (%)	Digestible intake
P1	47.8 ± 1.3^{a}	20.5 ± 0.8^{a}	57.2 ± 0.9^{a}	27.3 ± 0.8^{a}
P2	44.7 ± 0.3^{a}	20.5 ± 0.5^{a}	54.1 ± 1.2^{a}	24.2 ± 0.6^{b}
P3	45.5 ± 1.3^{a}	19.3 ± 0.7^{a}	57.5 ± 0.7^{a}	26.1 ± 0.7^{ab}
P4	45.2 ± 1.6^{a}	19.1 ± 0.6^{ab}	57.8 ± 0.9^{a}	26.2 ± 1.2^{a}
P5	42.6 ± 1.6^{b}	18.2 ± 0.7^{b}	57.4 ± 0.3^{a}	24.4 ± 0.9^{b}
P6	46.5 ± 1.0^{a}	18.7 ± 0.6^{b}	59.9 ± 0.5^{b}	27.8 ± 0.6^{ac}
P7	46.0 ± 1.0^{a}	16.3 ± 0.9^{c}	64.5 ± 1.9^{c}	29.7 ± 1.2^{c}
P8	34.6 ± 1.1^{c}	14.1 ± 0.6^{d}	59.2 ± 1.4^{ab}	20.5 ± 1.0^{d}
Mean	44.1 ± 0.7	18.3 ± 0.4	58.4 ± 0.5	25.8 ± 0.5
Range	31.0 - 52.6	12.3 - 23.3	49.9 - 69.6	17.8 - 34.1

a,b,c,d = statistical analysis code within columns ($P < 0.05$)

TABLE 4.6b: *Intake, excretion and apparent digestibility of dry matter by Brushtail Possums (values expressed as* $g.kgW^{-0.75}.d^{-1}$*) (Mean ± SE)*

Experiment	Intake	Faecal excretion	Digestibility (%)	Digestible intake
T1	39.7 ± 1.1^{b}	18.4 ± 1.2^{a}	53.9 ± 2.4^{a}	21.4 ± 0.9^{b}
T2	37.2 ± 0.8^{ab}	20.0 ± 1.0^{a}	46.3 ± 1.8^{b}	17.2 ± 0.5^{a}
T3	33.3 ± 2.7^{a}	16.8 ± 2.1^{ab}	50.1 ± 2.6^{a}	16.6 ± 0.6^{a}
T4	30.0 ± 2.8^{a}	15.2 ± 1.5^{b}	49.3 ± 2.0^{ab}	14.8 ± 1.6^{a}
T5	32.1 ± 1.2^{a}	16.0 ± 0.9^{b}	50.3 ± 1.1^{a}	16.1 ± 0.4^{a}
Mean	35.5 ± 1.1	17.4 ± 0.6	50.9 ± 1.0	18.1 ± 0.7
Range	24.5 - 45.4	12.7 - 23.7	43.4 - 59.0	11.8 - 23.3

a,b = statistical analysis code within columns ($P < 0.05$)

TABLE 4.7a: *Intake, excretion and digestibility of cell contents by Greater Gliders (values as $g.kgW^{-0.75}.d^{-1}$) (Mean ± SE)*

Experiment	Intake	Faecal excretion	Digestibility (%)	Digestible intake
P1	30.7 ± 0.9^{ab}	9.5 ± 0.3^{a}	68.9 ± 0.9^{c}	21.1 ± 0.7^{b}
P2	26.9 ± 0.7^{c}	9.4 ± 0.3^{ab}	64.9 ± 1.0^{d}	17.4 ± 0.3^{c}
P3	30.3 ± 0.2^{b}	$9.8 + 0.3^{a}$	67.7 ± 1.2^{cd}	20.6 ± 0.5^{b}
P4	32.0 ± 0.8^{a}	7.8 ± 0.5^{c}	75.6 ± 1.4^{a}	24.2 ± 0.8^{a}
P5	29.1 ± 1.1^{b}	8.4 ± 0.3^{bc}	71.2 ± 0.2^{b}	20.7 ± 0.8^{b}
P6	31.3 ± 0.7^{a}	8.6 ± 0.2^{b}	72.7 ± 0.5^{ab}	22.8 ± 0.6^{a}
P7	32.3 ± 0.7^{a}	7.8 ± 0.5^{c}	75.8 ± 1.5^{a}	24.5 ± 0.8^{a}
P8	22.7 ± 0.9^{d}	6.6 ± 0.3^{d}	71.0 ± 1.0^{bc}	16.1 ± 0.7^{c}
Mean	29.4 ± 0.5	8.5 ± 0.2	71.0 ± 0.6	20.9 ± 0.5
Range	20.1 - 34.5	5.5 - 10.8	60.9 - 79.9	14.6 - 27.3

a,b,c,d = statistical analysis code within columns ($P < 0.05$)

TABLE 4.7b: *Intake, excretion and digestibility of cell contents by Brushtail Possums (values expressed as $g.kgW^{-0.75}.d^{-1}$) (Mean ± SE)*

Experiment	Intake	Faecal excretion	Digestibility (%)	Digestible intake
T1	28.0 ± 0.8^{b}	10.9 ± 0.6^{a}	61.0 ± 1.6^{a}	17.0 ± 0.6^{b}
T2	27.3 ± 0.6^{ab}	11.5 ± 0.5^{a}	57.8 ± 1.1^{a}	15.8 ± 0.2^{ab}
T3	24.1 ± 1.9^{a}	9.7 ± 1.5^{ab}	60.3 ± 3.3^{a}	14.4 ± 0.4^{a}
T4	21.6 ± 2.0^{a}	8.5 ± 1.1^{c}	60.7 ± 2.7^{a}	13.1 ± 1.2^{a}
T5	22.9 ± 0.9^{a}	8.8 ± 0.7^{bc}	61.6 ± 1.5^{a}	14.0 ± 0.2^{a}
Mean	25.4 ± 0.8	10.1 ± 0.4	60.5 ± 0.9	15.3 ± 0.5
Range	17.6 ± 32.1	6.9 - 13.4	52.7 - 66.4	10.7 - 18.7

a,b,c = statistical analysis code within columns ($P < 0.05$)

(b) **Feeding Experiments:** Greater Gliders: A total of eight balance experiments were performed during different seasons of the year (Table 4.5) and data were collected on the intake, excretion and digestibility of various foliage components. Seasonal comparisons were made between summer (P1&P8) and winter (P5&P6) experiments.

Brushtail Possums: The difficulties encountered in maintaining Brushtail Possums on foliage diets restricted both the number and timing of the feeding experiments. Five experiments were carried out in winter, spring and summer (Table 4.5), although the foliage offered was always the oldest mature leaves on the tree. Seasonal comparisons were again made between summer and winter experiments even though the oldest foliage was always offered.

Animals of both species were housed in metabolism cages (Figure 2.1) for at least three weeks prior to the feeding experiment and feed intake was stable for at least 14 days prior to the seven day collection periods. Details of the housing, light regime and feeding and collection procedures have been given in Section 2.3. The analytical and statistical techniques applied to samples and data were described in Sections 2.4 and 2.5 respectively.

4.6 **Results**

4.6.1 **Dry matter and cell contents**

Table 4.6a shows the intake, excretion and digestibility of dry matter in Greater Gliders while Table 4.6b shows the same set of measurements for the Brushtail Possums. Parameters of the digestibility of cell contents for the two species are given in Tables 4.7a and 4.7b. There were no significant differences between summer and winter experiments in the intake, excretion and digestibility of dry matter or cell contents in either species. There was no significant relationship between dry matter digestibility and dry matter intake in either species. The intake of apparently digestible cell contents by Greater Gliders increased ($P<0.001$)

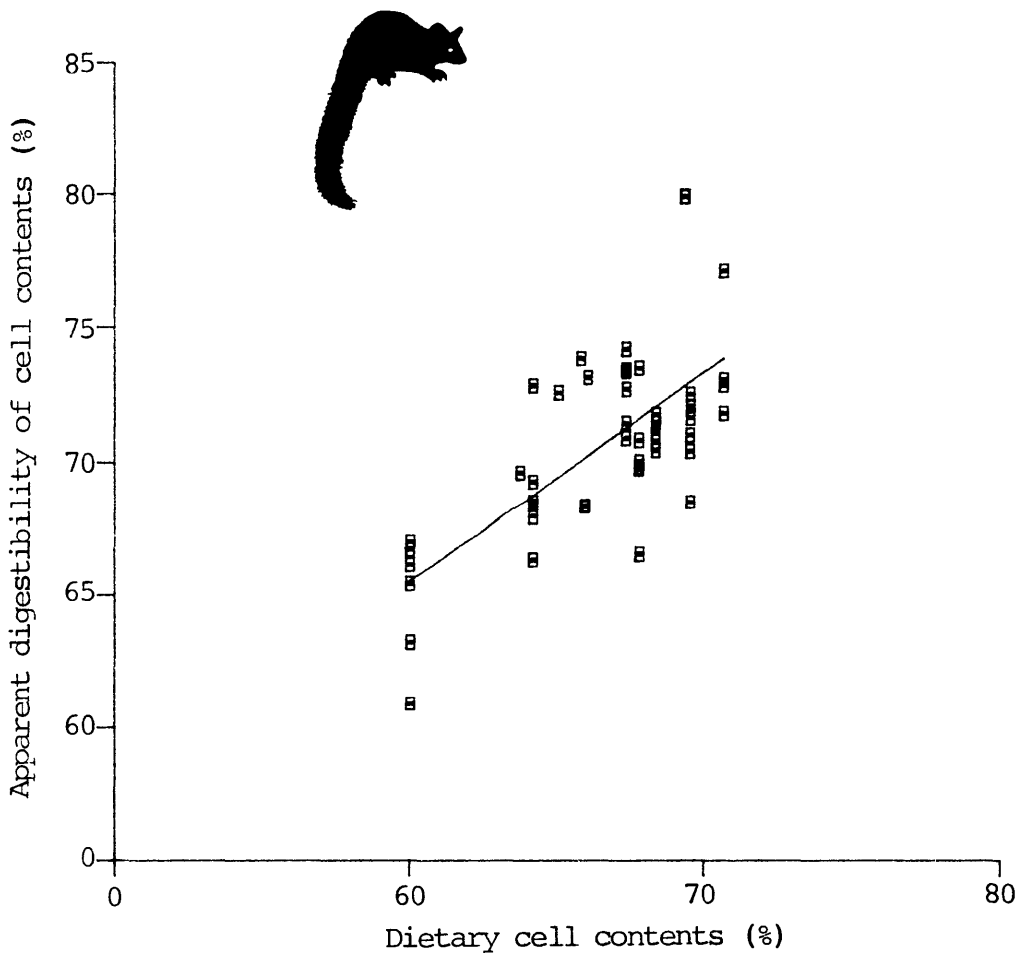


FIGURE 4.2: *Relationship between concentration of cell contents in the diet and apparent digestibility of cell contents by Greater Gliders.*

Regression equation:
$y = 18.39 + 0.785x$, $r = 0.687$ ($P<0.001$), RSD = 2.60

TABLE 4.8a: *Intake, faecal excretion and apparent digestibility of E. radiata cell wall constituents by Greater Gliders (values expressed as* $g.kg^{-0.75}.d^{-1}$*) (Means ± SE)*

Constituent	Experiment	Intake	Faecal excretion	Digestibility (%)	Digestible intake
NEUTRAL DETERGENT FIBRE	P1	17.1 ± 0.5^{c}	10.9 ± 0.5^{a}	36.2 ± 1.4^{a}	6.2 ± 0.2^{bc}
	P2	17.9 ± 0.1^{c}	11.1 ± 0.3^{a}	38.0 ± 1.7^{a}	6.8 ± 0.3^{c}
	P3	14.4 ± 0.1^{abc}	10.7 ± 0.3^{a}	25.5 ± 1.8^{b}	3.7 ± 0.3^{d}
	P4	14.0 ± 0.3^{a}	8.5 ± 0.4^{c}	39.0 ± 3.0^{a}	5.5 ± 0.5^{ab}
	P5	13.5 ± 0.5^{a}	9.8 ± 0.4^{b}	27.5 ± 1.1^{b}	3.7 ± 0.2^{d}
	P6	15.2 ± 0.3^{b}	10.1 ± 0.4^{ab}	33.5 ± 1.2^{ab}	5.1 ± 0.1^{a}
	P7	13.7 ± 0.4^{a}	8.5 ± 0.4^{cd}	37.8 ± 3.1^{a}	5.2 ± 0.5^{a}
	P8	12.0 ± 0.4^{d}	7.5 ± 0.3^{d}	36.8 ± 2.2^{a}	4.4 ± 0.3^{ad}
	Mean	14.7 ± 0.3	9.6 ± 0.2	34.3 ± 1.0	5.1 ± 0.2
	Range	10.8-18.6	6.8-12.6	17.8-48.5	2.6-7.8
HEMI-CELLULOSE	P1	1.4 ± 0.1^{b}	0.1 ± 0.1^{ab}	96.6 ± 10.0^{a}	1.3 ± 0.1^{a}
	P2	-0.4 ± 0.1^{e}	-0.2 ± 0.2^{b}	47.5 ± 49.2^{a}	-0.1 ± 0.2^{c}
	P3	1.8 ± 0.0^{c}	0.6 ± 0.1^{ab}	67.7 ± 3.2^{a}	1.2 ± 0.1^{a}
	P4	1.3 ± 0.0^{b}	0.4 ± 0.1^{a}	73.4 ± 6.2^{a}	1.0 ± 0.1^{a}
	P5	0.8 ± 0.0^{a}	0.3 ± 0.1^{a}	67.2 ± 13.1^{a}	0.5 ± 0.1^{b}
	P6	0.2 ± 0.0^{d}	0.1 ± 0.1^{a}	48.2 ± 77.2^{a}	0.1 ± 0.1^{c}
	P7	0.8 ± 0.2^{a}	0.3 ± 0.2^{a}	76.1 ± 14.7^{a}	0.5 ± 0.1^{b}
	P8	0.7 ± 0.1^{a}	0.2 ± 0.1^{a}	72.1 ± 7.3^{a}	0.5 ± 0.1^{b}
	Mean	0.8 ± 0.1	0.2 ± 0.1	68.6 ± 11.2	0.6 ± 0.1
	Range	-0.4-1.9	-0.9-0.9	-274.0-215.7	-0.9-1.8

ACID DETERGENT FIBRE	P1	15.7 ± 0.4^{bc}	10.9 ± 0.5^{ad}	31.0 ± 2.1^{ab}	4.9 ± 0.3^{a}
	P2	18.3 ± 0.1^{d}	11.3 ± 0.4^{d}	38.2 ± 2.2^{a}	7.0 ± 0.4^{d}
	P3	12.6 ± 0.1^{a}	10.1 ± 0.2^{a}	19.4 ± 1.7^{c}	2.4 ± 0.2^{c}
	P4	12.6 ± 0.3^{a}	8.2 ± 0.4^{bc}	35.4 ± 2.9^{a}	4.5 ± 0.4^{a}
	P5	12.7 ± 0.5^{a}	9.5 ± 0.3^{bc}	25.0 ± 1.2^{bc}	3.2 ± 0.2^{bc}
	P6	15.0 ± 0.3^{b}	10.0 ± 0.4^{ac}	33.4 ± 1.3^{a}	5.0 ± 0.1^{a}
	P7	12.9 ± 0.3^{a}	8.2 ± 0.5^{b}	36.3 ± 3.8^{a}	4.7 ± 0.5^{a}
	P8	11.2 ± 0.4^{e}	7.3 ± 0.3^{e}	34.5 ± 2.2^{a}	3.9 ± 0.3^{ab}
	Mean	13.9 ± 0.3	9.4 ± 0.2	31.6 ± 1.2	4.4 ± 0.2
	Range	10.0-18.7	6.4-12.8	11.9-46.6	1.5-8.4
CELLULOSE	P1	8.1 ± 0.2^{b}	5.1 ± 0.4^{d}	37.5 ± 3.7^{c}	3.0 ± 0.3^{a}
	P2	8.7 ± 0.1^{b}	4.2 ± 0.3^{a}	52.1 ± 4.1^{b}	4.5 ± 0.4^{c}
	P3	6.7 ± 0.1^{a}	4.0 ± 0.2^{a}	39.4 ± 3.2^{a}	2.6 ± 0.2^{a}
	P4	6.4 ± 0.1^{a}	3.5 ± 0.2^{ab}	45.1 ± 3.4^{a}	2.9 ± 0.2^{a}
	P5	6.6 ± 0.3^{a}	3.9 ± 0.3^{a}	41.4 ± 3.0^{a}	2.7 ± 0.1^{a}
	P6	6.3 ± 0.1^{ac}	3.9 ± 0.2$_{bc}$	38.9 ± 2.4^{ac}	2.5 ± 0.1^{ab}
	P7	6.2 ± 0.2^{c}	3.1 ± 0.2^{bc}	49.9 ± 3.8^{ab}	3.1 ± 0.3^{a}
	P8	4.3 ± 0.1^{d}	2.5 ± 0.2^{c}	42.2 ± 3.9^{a}	1.8 ± 0.2^{b}
	Mean	6.7 ± 0.2	3.8 ± 0.1	43.3 ± 1.3	2.9 ± 0.1
	Range	4.0-8.9	2.1-6.2	26.8-66.2	1.1-5.8
LIGNIN	P1	7.6 ± 0.2^{f}	5.8 ± 0.3^{a}	24.0 ± 2.9^{a}	1.8 ± 0.2^{ab}
	P2	9.6 ± 0.1$_{h}$	7.1 ± 0.3^{d}	25.6 ± 3.0^{a}	2.5 ± 0.3^{a}
	P3	5.9 ± 0.0^{a}	6.1 ± 0.1^{a}	-3.0 ± 1.8^{b}	-0.2 ± 0.1^{c}
	P4	6.3 ± 0.2^{cd}	4.7 ± 0.2^{c}	25.5 ± 3.4^{a}	1.6 ± 0.2^{b}
	P5	6.0 ± 0.2^{bc}	5.6 ± 0.1^{ab}	6.9 ± 2.6^{b}	0.4 ± 0.2^{c}
	P6	8.7 ± 0.2^{g}	6.1 ± 0.3^{a}	29.3 ± 2.0^{a}	2.5 ± 0.2^{a}
	P7	6.7 ± 0.2^{dc}	5.2 ± 0.3^{b}	23.6 ± 3.7^{a}	1.6 ± 0.2^{b}
	P8	6.9 ± 0.3^{e}	4.8 ± 0.3^{bc}	29.3 ± 3.7^{a}	2.1 ± 0.3^{a}
	Mean	7.2 ± 0.2	5.7 ± 0.2	20.1 ± 1.9	1.5 ± 0.2
	Range	5.6-9.8	3.9-8.3	-9.0-44.8	-0.5-3.6

with increasing concentration of cell contents in *E. radiata* (Figure 4.2). There was no significant relationship between these two parameters in the Brushtail Possum.

4.6.2 **Fibre digestibility**

(a) **Greater Gliders**

Data on the intake, faecal excretion and apparent digestibility of the fibre components of *E. radiata* foliage by Greater Gliders are presented in Table 4.8a. The digestibility of NDF was higher ($P<0.01$) in the summer experiments than in winter but there were no significant differences in the intake or digestibility of other fibre components between seasons. There was no significant relationship between fibre digestibility and dry mattter intake.

The mean NDF digestibility was 34.3 ±1.0% and of ADF 31.6 ± 1.2%. Although 20.1 % of the acid-detergent lignin was apparently digested there was wide variation between experiments with negative lignin digestibility being recorded in Experiment P3 (Winter).

Some of the problems inherent in the determination of cellulose and hemicellulose by difference have already been mentioned (Section 4.4). Although the intake and digestibility of cellulose was reasonably consistent between animals within experiments and between experiments, there was a wide range in the corresponding values for hemicellulose with intakes being negative in some experiments. Because of this variation, there were no significant differences in hemicellulose digestibility between experiments. The range of values for hemicellulose digestibility was -274 to +215 %.

(b) **Brushtail Possums**

Data on the intake, faecal excretion and digestibility of components of the cell walls of *E. melliodora* foliage by Brushtail Possums

TABLE 4.8b: *Intake, faecal excretion and apparent digestibility of E. melliodora cell wall constituents by Brushtail Possums (values expressed as* $g.kg^{-0.75}.d^{-1}$) *(Mean ± SE)*

Constituent	Experiment	Intake	Faecal excretion	Digestibility	Digestible intake
NEUTRAL DETERGENT FIBRE	T1	11.7 ± 0.3^{b}	7.4 ± 0.6^{a}	36.8 ± 4.9^{c}	4.3 ± 0.6^{c}
	T2	9.9 ± 0.2^{a}	8.4 ± 1.4^{ab}	14.6 ± 3.7^{b}	1.4 ± 0.4^{a}
	T3	9.2 ± 0.7^{a}	7.1 ± 0.6^{a}	23.5 ± 0.8^{a}	2.2 ± 0.2^{ab}
	T4	8.5 ± 0.8^{a}	6.7 ± 0.5^{a}	20.4 ± 2.3^{ab}	1.8 ± 0.3^{a}
	T5	9.2 ± 0.4^{a}	7.2 ± 0.3^{a}	22.4 ± 0.8^{a}	2.1 ± 0.1^{a}
	Mean	10.1 ± 0.4	7.4 ± 0.2	26.5 ± 2.4	2.8 ± 0.3
	Range	6.9-13.4	5.8-9.4	9.2-47.2	1.0-5.9
HEMI-CELLULOSE	T1	2.1 ± 0.3^{ab}	1.0 ± 0.2^{a}	51.1 ± 7.2^{ab}	1.1 ± 0.2^{ab}
	T2	1.8 ± 0.0^{a}	1.3 ± 0.2^{a}	29.3 ± 10.0^{ac}	0.5 ± 0.2^{a}
	T3	1.4 ± 0.2^{a}	0.8 ± 0.1^{a}	37.9 ± 11.4^{a}	0.6 ± 0.2^{a}
	T4	1.5 ± 0.1^{a}	1.0 ± 0.1^{a}	26.7 ± 6.3^{c}	0.4 ± 0.1^{a}
	T5	1.9 ± 0.1^{a}	0.9 ± 0.1^{a}	49.8 ± 4.8^{a}	0.9 ± 0.1^{a}
	Mean	1.8 ± 0.1	1.0 ± 0.1	41.9 ± 4.0	0.8 ± 0.1
	Range	0.7-2.7	0.5-1.7	9.3-70.8	0.2-1.7

ACID DETERGENT FIBRE	T1	9.7 ± 0.4^{bc}	6.4 ± 0.4^{ab}	32.9 ± 5.1^{b}	3.2 ± 0.6^{b}
	T2	8.1 ± 0.2^{ab}	7.2 ± 0.6^{b}	11.4 ± 3.1^{a}	0.9 ± 0.3^{a}
	T3	7.8 ± 0.7^{a}	6.2 ± 0.6^{a}	20.5 ± 3.2^{a}	1.6 ± 0.2^{a}
	T4	7.0 ± 0.7^{a}	5.7 ± 0.4^{a}	19.1 ± 2.4^{a}	1.4 ± 0.3^{a}
	T5	7.4 ± 0.3^{a}	6.2 ± 0.5^{a}	15.5 ± 0.8^{a}	1.1 ± 0.0^{a}
	Mean	8.3 ± 0.3	6.4 ± 0.2	22.8 ± 2.4	2.0 ± 0.3
	Range	5.7-11.7	4.9-8.3	7.5-46.7	0.6-5.6
CELLULOSE	T1	5.7 ± 0.3^{a}	3.2 ± 0.3^{a}	42.8 ± 6.9^{a}	2.5 ± 0.5^{b}
	T2	4.8 ± 0.1^{a}	3.8 ± 0.2^{b}	20.9 ± 4.4^{b}	1.0 ± 0.2^{a}
	T3	5.0 ± 0.4^{a}	3.5 ± 0.4^{a}	29.5 ± 5.6^{a}	1.5 ± 0.3^{ab}
	T4	4.5 ± 0.4^{ab}	3.1 ± 0.2^{a}	30.9 ± 3.4^{a}	1.4 ± 0.3^{a}
	T5	4.2 ± 0.2^{b}	3.6 ± 0.4^{ab}	14.9 ± 0.8^{b}	0.6 ± 0.0^{a}
	Mean	5.0 ± 0.1	3.3 ± 0.1	31.5 ± 3.1	1.7 ± 0.2
	Range	3.7-7.6	2.3-4.1	12.3-57.9	0.6-4.4
LIGNIN	T1	4.0 ± 0.1^{c}	3.2 ± 0.2^{a}	18.9 ± 3.8^{a}	0.8 ± 0.2^{c}
	T2	3.3 ± 0.1^{a}	3.4 ± 0.2^{a}	-2.0 ± 4.7^{b}	-0.1 ± 0.2^{a}
	T3	2.9 ± 0.3^{ab}	2.7 ± 0.3^{a}	5.5 ± 2.3^{b}	0.2 ± 0.1^{ab}
	T4	2.6 ± 0.3^{b}	2.6 ± 0.3^{a}	-2.0 ± 7.6^{b}	0.0 ± 0.2^{a}
	T5	3.2 ± 0.1^{a}	2.6 ± 0.9^{a}	16.2 ± 1.1^{a}	0.5 ± 0.0^{bc}
	Mean	3.4 ± 0.2	3.0 ± 0.2	9.4 ± 3.2	0.3 ± 0.1
	Range	2.0-4.5	2.1-5.2	-15.2-32.1	-0.7-1.2

are given in Table 4.8b. No seasonal trends were apparent. The mean NDF digestibility was 26.5 ± 2.4% and the mean ADF digestibility was 22.8 ± 2.4%. Hemicellulose intake and digestibility was less variable than in the Greater Gliders and this could probably be attributable to the higher proportion of dietary hemicellulose in *E. melliodora* foliage. On the other hand, lignin digestibility was more variable both within and between experiments than in Greater Gliders and negative digestibilities were recorded in two of the five experiments.

4.7 **Discussion**

4.7.1 **Dietary bias**

Before discussing the results obtained, it is important to note that it is possible that the choice of the tree species used as diets in this study may have led to some bias in the results. *E. radiata* was a common component of the forests in which all Greater Gliders were captured and is probably one of the two principal dietary items of Greater Gliders wherever the ranges of this eucalypt species and the animal overlap. Several unusual features of the foliage of *E. radiata* (e.g. high essential oil content) may have had considerable impact on some aspects of the physiology of Greater Gliders but these are probably features to which free-living animals are exposed.

On the other hand, Brushtail Possums rarely eat a sole diet of eucalypt leaf in the wild. In this study, the acceptance of *E. melliodora* foliage was very variable; the majority of trees offered were completely rejected, others were sampled and only a few were readily eaten. However, since the the aim of the study was to examine the utilization of eucalypt foliage by the two herbivores, it was deemed important that both be fed on a single species, albeit different species and in the case of the Brushtails, a species which is consumed to an unknown extent in the wild.

TABLE 4.9: *Intake and digestibility of dry matter and digestibility of cell walls in marsupials fed eucalypt foliage diets*

	Ringtail Possum (Chilcott and Hume, 1984a)	Greater Glider (this study)	Brushtail Possum (this study)	Koala (Cork *et al.*, 1983)
Body weight (kg)	0.7	1.1	2.3	7.5
Diet	*E. andrewsii*	*E. radiata*	*E. melliodora*	*E. punctata*
Dry matter intake ($g.kg^{-0.75}.d^{-1}$)	42	44	36	41
Digestibility (%) of dry matter	59	58	51	54
Digestibility (%) of NDF	45	34	27	25
Digestibility (%) of ADF	44	32	23	25
Digestibility (%) of Hemi-cellulose	48	69	42	24
Digestibility (%) of Cellulose	59	43	32	31
Digestibility (%) of Lignin	26	20	9	19

4.7.2 Intake and digestibility of dry matter and cell contents

The voluntary dry matter intake of adult Greater Gliders fed *E. radiata* foliage was 44g·kgW$^{-0.75}$·d^{-1}. The only previous estimate of dry matter intake was made indirectly by Marples (1973), based on the mass and energy content of stomach digesta from wild-shot animals. This estimate was only 20g·d^{-1}. The present measurements were direct and thus must be more reliable than those of Marples (1973). The most likely source of error in Marples' study is the assumption that the rate of disappearance of dry matter from the stomach during the non-feeding period was the same as that during the active feeding period. On the basis of results from other species, rate of movement out of the stomach is likely to be greatly increased during feeding. Alternatively, if high energy-content compounds such as essential oils were either absorbed from the stomach (Igimi *et al.*, 1974, Chapter 7) or lost during mastication or during oven drying, then the dietary energy content and hence the dry matter intake would have been underestimated.

The intake of dry matter of *E. melliodora* by Brushtail Possums was only 36g·kgW$^{-0.75}$·d^{-1} which is lower than that of other small folivores fed eucalypt leaf (Table 4.9). Few data exist on the intake of eucalypt leaf by Brushtail Possums. Lintermans (1981) measured intakes of only 8g·kgW$^{-0.75}$·d^{-1} in animals offered *E. viminalis* foliage. The diets were clearly unacceptable and the animals lost 20% of their initial body mass over 14 days. Although the dry matter intakes of the semi-purified diets used by Wellard and Hume (1981b) were similar to the values recorded here, the higher digestibility of these diets resulted in higher intakes of digestible dry matter.

Digestibility of the dry matter of the two foliages by the two species was similar to values recorded for other marsupial folivores (Table 4.9). Higher digestibilities of the dry matter of semi-purified diets were recorded by Wellard and Hume (1981b) (mean = 79%) at similar levels of dietary NDF, but the low degree of lignification of the fibre source used may partly explain the differences between this and the present study.

TABLE 4.10: *Digestibility of the dry matter and fibre of foliage or browse diets by some herbivores*

Species	Diet and composition (%) Item	NDF	Lignin	Digestibility (%) Dry matter	NDF	ADF	Cellulose	Reference
FOREGUT FERMENTERS								
Deer	Mixed tree foliage	47-54 (42-65)[1]	12-13 (5-8)	37-41 (54-55)	18-21 (37-58)	- -	- -	Robbins *et al.* (1975) Robbins *et al.* (1975)
Goats	Tree foliage (5 spp.)	34-62 (49)[1]	10-14 (8)	35-60 (63)	7-38 (47)	4-21 (37)	- -	Wilson (1977)
Sheep	Tree foliage (3 spp.)	44-61 (49)[1]	10-14 (8)	29-43 (63)	1-28 (47)	7-14 (40)	-	Wilson (1977)
Elk	Maple (*Acer*) foliage	33 (40-73)[1]	8 (5-7)	41 (50-55)	30 (51-55)	27 (50-52)	- -	Mould and Robbins (1982)
Colobus	Foliage (exotic)	15[5]		40-60[2]	-	-	40-60[2]	Kay *et al.* (1976)
HINDGUT FERMENTERS								
Howler Monkey	87% young leaves, 13% fruit	33-54	4-26	48	37-45	37-45	68	Milton *et al.* (1980) Nagy and Milton (1979)
Mountain Hare	Willow, birch, heather twigs	38-49[3]		18-41	-	9-28	-	Pehrson (1981, 1983)
Snowshoe Hare	Red maple (*Acer* spp.)[4]	27[3]		45-48	-	2-12	-	Holter *et al.* (1974)
Koala	*E. punctata* Mixed eucalypt leaf	34 24-27	14 5-8	54 54-70[2]	25 23-57[2]	25 9-55[2]	31 -	Cork *et al.* (1983) Ullrey *et al.* (1981b)
Brushtail Possum	*Pseudopanax arboreum* *E. melliodora*	7[5] 29	10 10	57 51	- 27	- 23	35 32	Fitzgerald (1978) Present study
Ringtail Possum	*E. andrewsii*	51	20	59	45	44	59	Chilcott (1982)
Greater Glider	*E. radiata*	33	17	58	34	32	43	Present study

[1] Values in brackets are for herbage diets fed in same series of experiments; [2] Estimates made using lignin ratio method; [3] Acid detergent fibre; [4] Ground and pelleted foliage supplemented with concentrates; [5] Cellulose

The cell contents fraction consists of proteins, lipids, nonstructural carbohydrates (sugars and starches) and soluble phenolics. In most forages, the true digestibility of this fraction is considered to be close to 100% (Parra, 1978, Van Soest, 1982), the major endogenous faecal losses being bacteria and cellular debris. However, Mould and Robbins (1982) showed that the true digestibility of cell contents in Elk was depressed for forages containing significant quantities of soluble phenolics. In the present study, the significant relationship between the intake of apparently digestible cell contents and the content in the diet of the Greater Glider allowed the estimation of true digestibility and endogenous losses. However, the very narrow range of values for dietary cell contents (60-65%) in this study requires that some caution be exercised in interpreting the results.

The true digestibility of cell contents (78.5%) was lower than the values of 100% commonly reported for domestic species (Parra, 1978), but endogenous losses (18.4 g·100g intake) were higher and similar to values reported for deer and Elk (Mould and Robbins, 1982). Endogenous excretion of nitrogen in the Greater Glider (estimated in Chapter 5) was 0.22 g $N \cdot kgW^{-0.75} \cdot d^{-1}$. If we assume that bacteria are about 60% protein (Hungate, 1966), then the endogenous excretion of nitrogen represents about 3g of bacterial dry matter per day. It is likely that phenolic material in the faeces comprised a significant proportion of the remaining fraction and was the primary cause of the low true digestibility of cell contents. The relationship between apparently digestible intake and content of cell solubles in the diet of the Brushtail Possum was not significant. However, some comments on the effect of phenolics on the digestion of this fraction in the Brushtail will be made in Chapter 6.

4.7.3 **Fibre digestibility**

The lack of uniformity of particle size of feed and faecal samples may affect estimates of the digestibility of detergent fibre fractions. Although both diet and faeces samples were ground to pass the same 1.0mm sieve the majority of faecal particles were already smaller than this (Chapter 3). Ehle (1982) has shown that the NDF content in lucerne

decreases with decreasing particle size of the material used for analysis. While it is unlikely that this effect invalidates comparisons between the Greater Glider and the Brushtail Possum, it does suggest that fibre digestibility may have been overestimated in this study. It was not possible to estimate the magnitude of this error.

The extent of digestibility of the fibre of both eucalypt species was low when compared with the digestion of the cell walls of grass and herbage diets by foregut and hindgut fermenters (Parra, 1978, Cork, 1981, Van Soest, 1982, Hume, 1982). However, fibre digestibilities were similar to those found in herbivore species fed foliage or browse diets (Table 4.10). For example Mountain Hares fed twigs of willow, blueberry or heather digested only 25-30% of the ADF (Pehrson, 1983a). Similarly, the digestibility of browse diets by ruminants is markedly lower than that of herbage at similar levels of NDF (Table 4.10). There are several possible explanations for the low digestibility of the fibre of foliage or browse diets.

Lignin is the major cell wall component associated with low digestibility of herbivore diets (Parra, 1978, Van Soest, 1982, Jung and Fahey, 1983) although the mechanisms involved in this effect are as yet uncertain. There are three main theories - physical encrustation, effects on microbes and gut enzymes and the nature of lignin-carbohydrate complexes. The encrustation theory is supported by the finding that the true digestibility of cell contents in the Greater Glider was less than 100%. In contrast, the fact that the lignin content of grasses can be half that found in legumes of the same digestibility (Van Soest, 1982) argues strongly against the simple encrustation of cell wall constituents as a reason for the effects of lignin on digestion. Secondly, lignins have been shown to inhibit microbial growth and enzymatic digestion (Jung and Fahey, 1983). However, the problem with this theory is in using results obtained with isolated, and hence degraded, lignins, to predict effects in intact plant tissues (Van Soest, 1982). Finally, the occurrence of lignin-carbohydrate complexes resistant to celluloytic enzymes may also explain the effects of lignin on digestion. The increased digestibility of alkali treated grass straws (Van Soest, 1982) may be due to the breaking of

ester linkages between lignin and carbohydrate. Whatever the mechanisms involved, it seems likely that the low digestibility of tree foliage and browse may be partly attributable to the high degree of lignification of the cell walls.

Whether the low fibre digestibilities recorded in this study truly reflect the maximum potential degree of fibre digestibility in both species is not known. Short *et al.* (1974) found that the maximal extent of fibre digestion of honeysuckle leaves in the rumen of goats was attained after only eight hours. However, scanning electron microscopic (SEM) observations in the present study (Part C) showed that although there was little mesophyll tissue remaining in the faeces of either species, bacterial degradation of the cell wall was still occurring.

Sadler (1982) has measured the digestibility *in vitro* of Greater Glider faeces collected in conjunction with Experiment P8 of this study. There was little or no digestion of the faecal material in rumen fluid from a goat fed chopped lucerne hay followed by acid-pepsin incubation, although minor amounts of faecal dry matter (5-7%) were solubilized when these treatments were reversed. The *in vitro* incubation of *Eucalyptus* foliage in the same study showed an initial rapid (4-6 hours) phase of digestion (presumably due to removal of cell contents), but the slope of the curve approached zero from about 16 up to 72 hours, suggesting that little of the fibre was being digested. Although rumen cellulolytic bacteria are sometimes regarded as being more "potent" than hindgut micro-organisms (Van Soest, 1982) it would be useful to observe the pattern of fermentation using *in vitro* preparations from the Greater Glider or Brushtail Possum caecal contents. However, the available evidence does suggest that the maximal fibre digestion was approached.

Several studies have concluded that ruminant microbial fermentation can be affected by plant essential oils (Nagy *et al.*, 1964; Nagy and Tengerdy, 1968; Oh *et al.*, 1967,1968) and the implications of these results have been extended to hindgut fermenters as well. The effect in ruminants seems to depend on the composition of the oil and both inhibitory and stimulatory responses have been noted. It has been

suggested that these effects are reduced or minimized in animals that have had regular exposure to plants containing these compounds (Oh *et al.*, 1967; Hume, 1982). For example, McKenzie (1978) suggested that the abundance of the caecal flora in the Koala showed that the bacteria are able to withstand the germicidal effects of the ingested leaf terpenes. However, the techniques used and conclusions drawn from these early studies have several shortcomings (See Section 1.4.2.1). In contrast, in a more recent and complete study, Pederson and Welch (1982) showed that there was no effect of previous monoterpene exposure on the ability of rumen micro-organisms to digest forages *in vitro*. Although the adaptability of rumen micro-organisms is well documented (Warner, 1962; Hungate, 1966), the important question seems to be whether in fact there is significant interaction between dietary essential oils and the microbial population. Narjisse (1981, in Welch *et al.*, 1982) found negligible amounts of monoterpenes in the rumens of sheep and goats four hours after direct infusions of 3g of the compounds. Similarly, Cluff *et al.* (1982) found that 80% of the expected level of monoterpenes had been lost from the rumen contents of wild mule deer feeding on sagebrush. The idea that there was only minor interaction between the microbial population of the hindgut and leaf terpenes was developed independently in this study and will be discussed in more detail in Chapter 7. However, in summary it is likely that there was little effect of leaf terpenes on microbial activity in the hindgut of the Greater Glider and Brushtail Possum. This was more likely to be due to a lack of interaction between the micro-organisms and dietary terpenes rather than to any adaptational effects.

4.7.4 **Lignin digestibility**

The extent of lignin digestibility by Greater Gliders in this study are similar to values reported for the Koala and the Ringtail Possum (Table 4.9). In contrast Brushtail Possums digested only 11% of the lignin. There are several problems in determining the nutritional significance of apparent lignin digestion. Firstly, lignin is a poorly defined chemical fraction and little is known of the nature and structure of eucalypt foliage lignins. Secondly, lignin degradation is thought to be an exclusively aerobic process. For example, Butler and

Buckerfield (1972) suggested that lignin digestion in termites occured in aerobic sites in the predominately anaerobic gut, although no particular region was specified.

Several other explanations have been advanced to explain positive lignin digestion. Van Soest (1982) suggested that the distribution of particle sizes in the faeces is such that a significant proportion of the faecal lignin is lost during filtration of the residue during the determination of 72% H_2SO_4 lignin. Butler and Buckerfield (1972) also suggested that the passage of wood lignin through the termite gut might make it more soluble in 72% H_2SO_4 without involving degradation. Gaillard and Richards (1975) and Neilson and Richards (1978) showed that soluble lignin-carbohydrate complexes ocurred in the bovine rumen. If these also occurred in the hindgut and were not recovered in the faeces, then positive apparent lignin digestibilities would result. The UV spectra of acetyl bromide-soluble foliage and faecal lignins (Figure 4.1) showed differences suggestive of an alteration of lignin structure during passage through the gut. Similar findings have been reported for sheep (McCampbell and Thomas, 1972). While it seems unlikely that the aromatic portion of the lignin molecule could provide a useable energy source, bacteria may be able to split and ferment the carbohydrate moieties from the core lignin.

Whatever the reason for the apparent positive lignin digestibilities, it is clear that the use of lignin as an internal marker in eucalypt feeding folivores is not satisfactory. Using this technique, Ullrey *et al.* (1981b) found that Koalas digested 23-57% of the NDF of a mixed foliage diet (Table 4.10). This is significantly higher than estimates made by total collection by Cork *et al.* (1983). Part of the difference may be due to the lower lignin content of the diets used by Ullrey *et al.* (1981b), but digestibilities will be underestimated if the lignin marker is digested (not overestimated as suggested by Chilcott and Hume 1984a) and the differences between the two studies would thus be even greater.

FIGURE 4.3: Scanning electron micrographs of intact eucalypt leaf and sections from stomach contents.

(a) *E. radiata*: Torn section showing palisade mesophyll. Xylem element protruding. x 200; Side = 450μ.

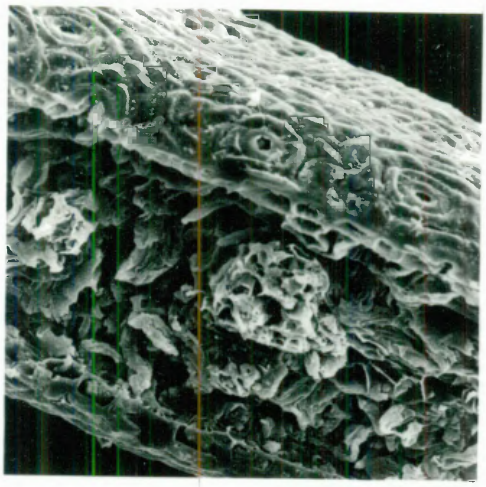
(b) *E. radiata*: Cut section showing spongy and palisade mesophyll and bundle sheath extensions. x360; 265μ.

(c) *E. melliodora*: Cut section showing palisade mesophyll and large sub-stomatal spaces. x260; 409μ.

(d) *E. melliodora*: Cut section showing palisade and spongy mesophyll surrounding a vascular bundle. x240; 420μ.

(e) Brushtail Possum - stomach content: Note epidermis and mesophyll tissue are predominately intact. x320; 300μ.

a



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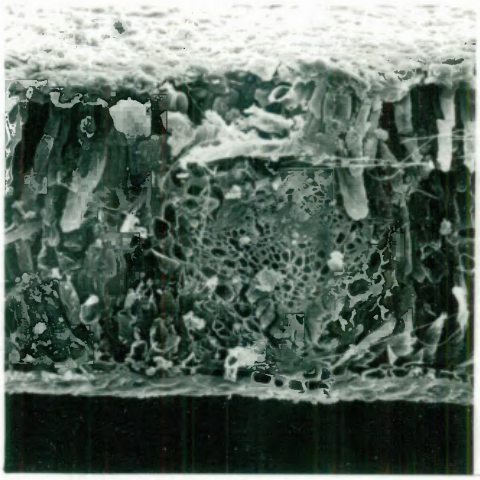

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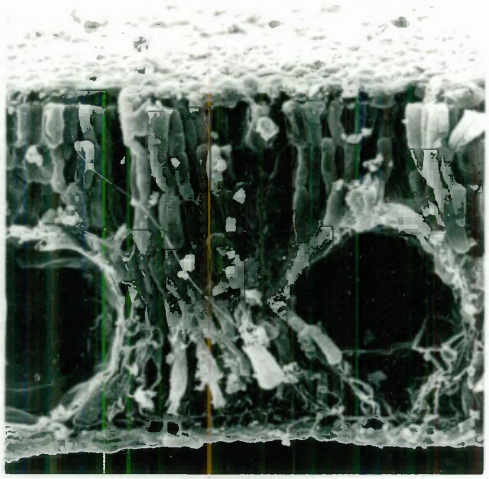

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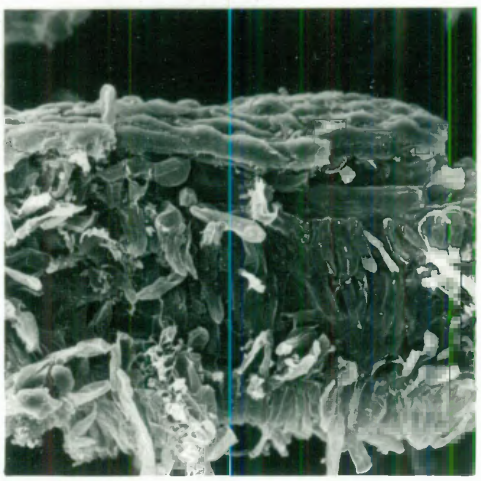


e

P A R T C

MICROSCOPIC EVALUATION OF FOLIAGE DIGESTION

4.8 Materials and Methods

Digesta and faeces fragments were collected from three Greater Gliders and three Brushtail Possums during the experiments described in Chapter 7. Samples of leaf were collected one day prior to the death of the animal; both species had been feeding on foliage from only one individual tree for at least 10 days previously. Digesta and faeces particles were separated by soaking in phosphate buffer (0.1M, pH 7.2) and sieving through a series of screens (0.25mm, 0.125mm, 0.075mm). Leaf sections were cut from fresh foliage after embedding in styrofoam. All material was fixed for 24 h in 3% glutaraldehyde in phosphate buffer (0.1M pH 7.2) and then thoroughly washed with the same buffer. Particles and leaf sections were then dehydrated in an aqueous ethanol series and dried by the critical point method after infiltration with CO_2 (Anderson, 1950). The samples were then mounted on brass stubs with double-sided adhesive tape and sputter coated with gold. The specimens were examined in a Jeol SM scanning electron microscope at an accelerating voltage of 15 keV.

4.9 Results

4.9.1 Plant ultrastructure in intact and degraded tissues

The transverse sections of both eucalypt species were similar. The lower epidermis of both species (Figures 4.3b - d) was papillated with stomates present on this surface only. There was a thick cuticle covering the epidermis of both species (Figures 4.3a - d). Surprisingly, no oil glands were seen in either species but both palisade and spongy mesophyll was present in both. Large numbers of vascular bundles and bundle sheath extensions (e.g. Figure 4.3b) showed the irregular wall thickening characteristic of collenchyma. This irregular thickening was probably of cellulose and hemicellulose. However the minor vein below the bundle in

FIGURE 4.4: Scanning electron micrographs of eucalypt fragments from the hindgut of the Greater Glider and Brushtail Possum.

(a) Brushtail Possum - Caecal contents: Mesophyll and mesophyll cell wall with a variety of attaching bacterial forms including rods, cocci and spirals. x4000; Side = 24μ.

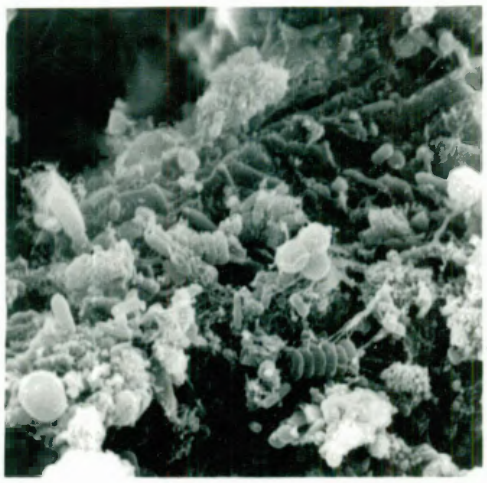
(b) Brushtail Possum - Caecal contents: Mesophyll cell wall showing high density of attaching bacterial rods and cocci. Low power of (d). x4000; 24μ.

(c) Brushtail Possum - Caecal content: Bacteria attaching to each other and to cell wall by threadlike extracellular material. x9400; 9μ.

(d) Brushtail Possum - Caecal content: Bacterial rods and cocci attaching to mesophyll cell wall by extracellular fibres. High power of (b). x18300; 5μ.

(e) Brushtail Possum - Caecal content: Epidermal surface with minimal attaching bacteria. x4000; 24μ.

(f) Greater Glider - Caecal content: Bacteria lying in a degraded zone on an unidentified cell wall. x7800; 11μ.



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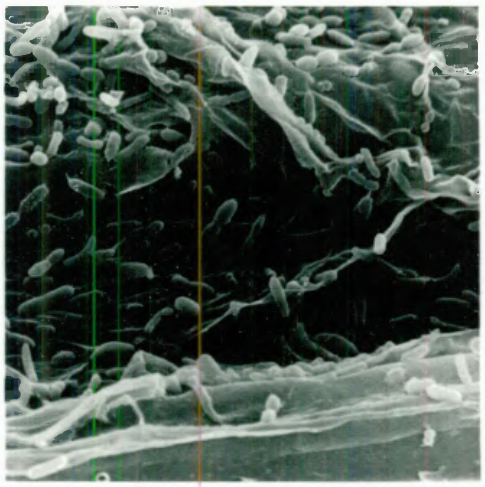

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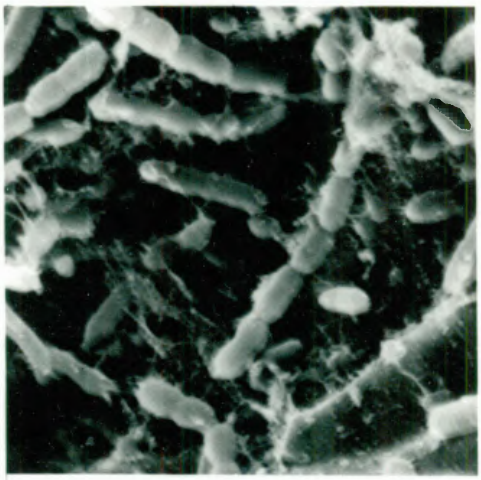

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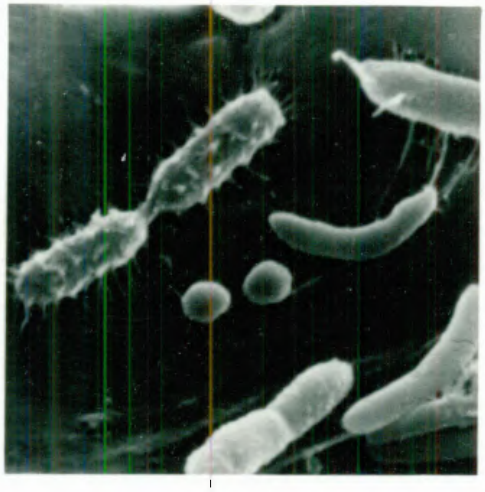

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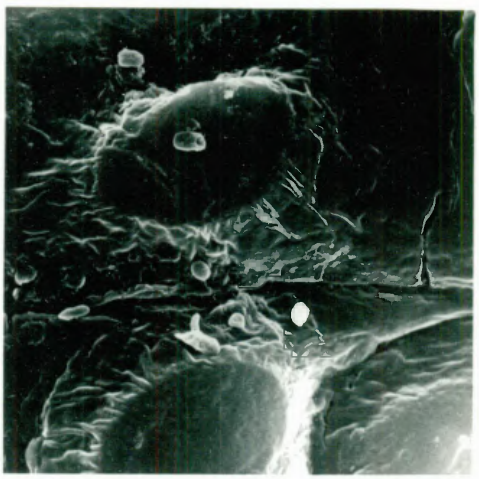

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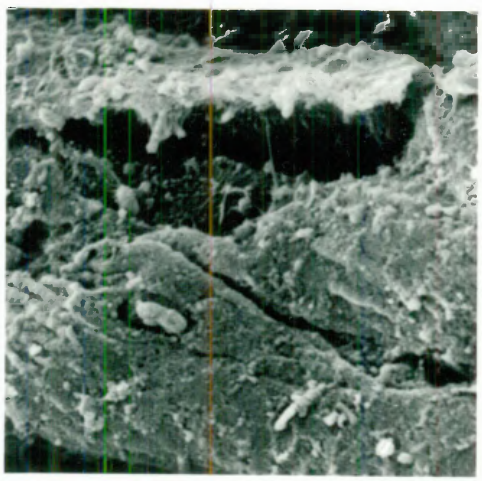

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FIGURE 4.5: Scanning electron micrographs of eucalypt fragments from the faeces of the Greater Glider and Brushtail Possum.

(a) Greater Glider - Faecal fragment: Note bacteria attaching to cellulose which overlies the cuticular skeleton. x10300; Side=8μ.

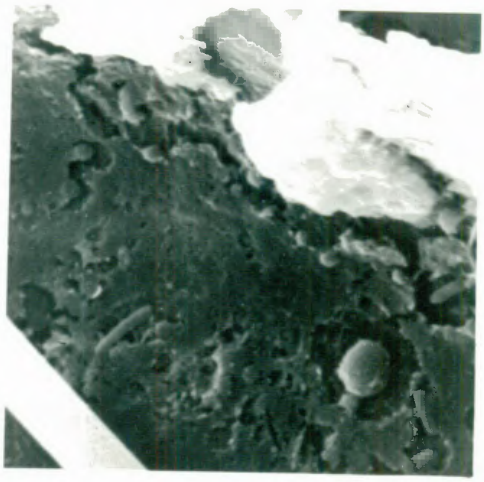
(b) Greater Glider - Faecal fragment: Bacterial rods and cocci degrading cellulose on underside of cuticular skeleton. x6000; 15μ.

(c) Greater Glider - Faecal fragment: Areas of digested palisade mesophyll. Cuticle and epidermis and bundle sheath extension are intact. Some areas of spongy mesophyll around the bundle also remain. x240; 490μ.

(d) Brushtail Possum - Faecal fragment: Partially degraded vascular bundle showing both spiral and pitted xylem elements. Low power of (e). x400; 237μ.

(e) Brushtail Possum - Faecal fragment: Calcium oxalate crystal lying between two spiral xylem elements. Bacteria attaching to oxalate crystal. High power of (d). x4000; 24μ.

(f) Greater Glider - Faecal fragment: Underside of cuticular skeleton. White rods are glass fibres from sample preparation. x2000; 47μ.



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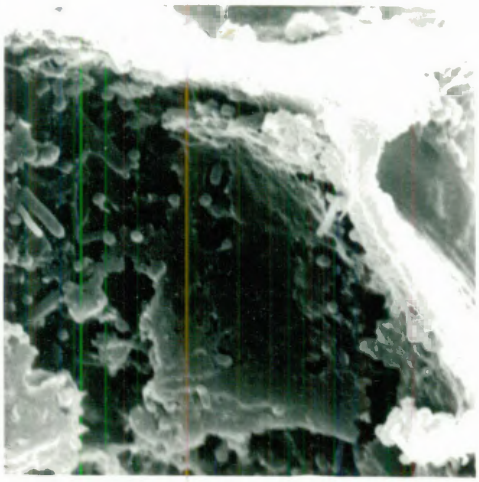

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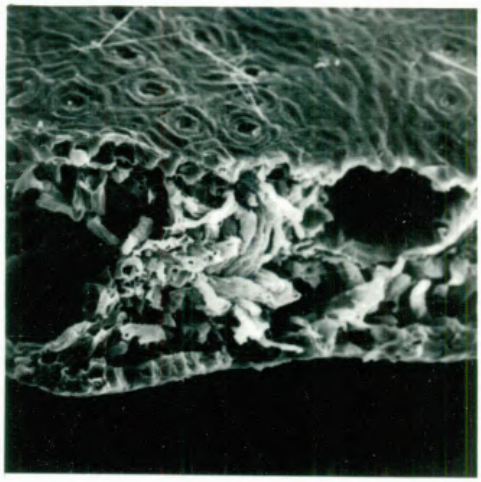

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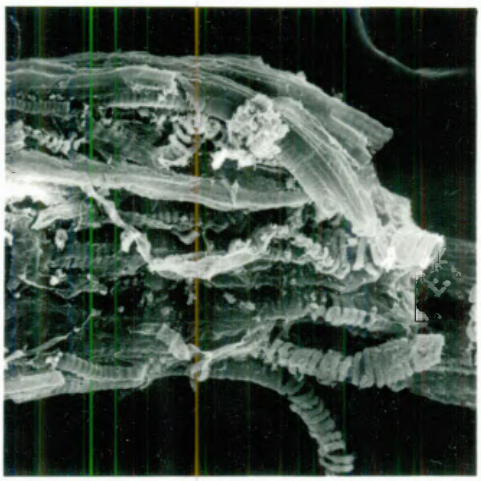

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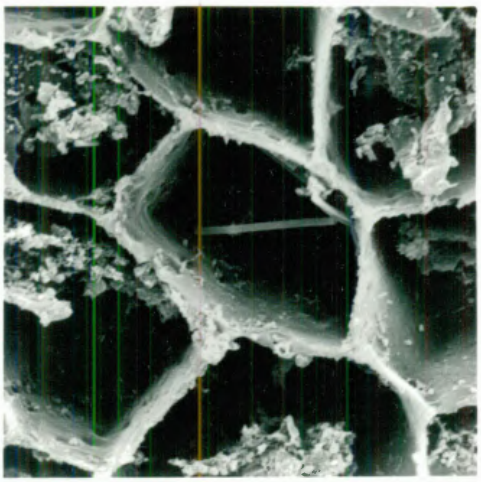

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this micrograph appeared to be extensively lignified. Several sections of *E. melliodora* (e.g. Figure 4.3c) showed distinct substomatal spaces surrounded by extensive areas of palisade mesophyll.

Fragments from the stomach contents (e.g. Figure 4.3e) of both species showed that most of the cuticle, epidermis and mesophyll tissue was intact. The most striking feature of the caecal and faecal material from each species was the abundance of very fine particles. Much of this material was highly degraded (e.g. Figures 4.5c - f) although several fragments showed areas of undigested mesophyll cells. For example, the fragment from the faeces of the Greater Glider shown in Figure 4.5c appeared to be little degraded. Small areas of spongy mesophyll are present in the bottom of the picture and the vascular tissue seemed to be relatively intact as were the minor areas of palisade mesophyll above. The epidermis and cuticle are similar to that seen in undigested tissues, suggesting that these tissues are very resistant to degradation.

There was a large amount of fibrous material in the faeces of both species. Figure 4.5d shows a vascular bundle from the faeces of the Brushtail Possum. Much of the cellulose appeared to have been digested, leaving only the more resistant spiral and pitted xylem elements. A cubic crystal can be seen lying between two spiral xylem elements in Figure 4.5d. Similar structures were also seen associated with epidermal skeletons. Epidermal skeletons such as that in Figure 4.5f may be the result of the intrusion of pegs of cuticular material into the underlying plant tissues.

4.9.2 **Micro-organisms involved with degraded plant tissues.**

Judging from the size of the observed micro-organisms (0.5μm to 10 μm), all were bacteria. Neither protozoa nor fungi were observed on any plant fragment. No bacteria were observed on either the cut leaf sections or on fragments from the stomach contents of either species. Figures 4.4a and 4.4b show large numbers of a mixed grouping of bacteria on mesophyll cell walls of a fragment from the caecum of the Brushtail Possum.

Importantly, all bacteria were observed at sites of physical damage to the plant surface. Figure 4.4e shows a high-power view of the lower epidermis of a fragment from the faeces of a Brushtail Possum. Very few bacteria can be seen on this surface compared with the mesophyll cell walls in Figures 4.4a and 4.4b. Similarly, few bacteria can be seen on the more highly lignified tissues such as the spiral vessel elements in Figure 4.5e.

Figure 4.4c shows large numbers of threadlike fibres linking bacteria to each other and to their substrate, in this case a mesophyll cell wall. Zones of degradation can be seen below some of the attaching bacteria (e.g. Figures 4.4f, 4.5a,b). Extracellular fibrous structures can be seen more clearly in Figure 3d extending from a single bacterium, and degraded zones can be seen under these bacteria as well.

4.10 **Discussion**

Microscopic observations have been used in several recent studies of digestion in herbivores to better evaluate the effect of plant structural features on digestibility. Many of these studies have used *in vitro* preparations for ease of sample preparation. However, some (eg. Akin *et al.*, 1974) have used rumen fluid incubation periods far in excess of that found in the intact animal. The use of digesta collected directly from the animal as in the present study avoids these sorts of problems.

Intact sections of both *E. radiata* and *E. melliodora* leaves showed an arrangement of tissues typical of that found in eucalypts (McLuckie and McKie, 1958). The cuticle and epidermis of both species was thick and robust in appearance with stomates present only on the lower surface. Although many large sub-stomatal spaces were seen in *E. melliodora* sections, they were absent from all sections of *E. radiata* examined. Therefore, it is likely that the large cavities seen in transverse sections of *E. radiata* collected from the faeces of Greater Gliders (e.g. Figure 4.5c) are digested mesophyll tissue rather than sub-stomatal spaces.

Both pitted and spiral xylem elements were apparent in the vascular bundles of both intact and degraded sections. Xylem elements are

found intact in the faeces of many herbivore species (e.g. Bauchop and Clarke, 1977) and are usually among the most highly lignified parts of the plant although in some grasses they are composed principally of silica (McManus *et al.*, 1979). Also associated with the vascular bundles were a large number of cubic crystals. These appeared very similar to calcium oxalate crystals from lucerne stems (Ward *et al.*, 1978). Calcium oxalate crystals are found associated with the vascular bundles of a wide range of other plants (Franceschi and Harner, 1980) (including *E. ovata* : Lomdahl, 1983) and may be responsible for the low calcium availability to herbivores of these plants (McKenzie *et al.*, 1981). Calcium crystals may also impede access of the micro-organisms to the underlying tissues. However, micro-organisms capable of degrading oxalate are widely known (Allison and Cook, 1981) and the pitting on the surface of the crystal in Figure 4.5e suggests that microbial degradation of oxalate may also occur in the Brushtail Possum at least.

It was not possible to determine the sequence of digestion of tissues in this study. However, the absence of mesophyll in most faecal sections and the apparent resistance of the highly lignified tissues suggests that the mesophyll and phloem was most readily digested. Akin *et al.* (1975) and Harbers *et al.* (1981) have shown a similar pattern of tissue degradation in ruminants and horses respectively. In both these studies the cuticle remained intact until digestion of the underlying tissues caused its collapse. This did not occur in the present study since there appeared to be a skeleton of highly lignified or suberinized epidermal cells under the cuticle. Consequently, the cuticle remained intact through all stages of the digestive process.

Bacteria were the only micro-organisms seen on any plant particle. There was a dense population of a diverse range of bacteria adhering to plant particles in the caecum /proximal colon and faeces of both species confirming that the hindgut was the principal site of microbial digestion. Several different morphological types were observed including rods, cocci and organisms resembling actinomycetes (Akin, 1976). Little is known of the hindgut flora of arboreal marsupials. McKenzie (1978) reported a dense mat of bacteria adhering to the caecal epithelia of the Koala. London (1981)

made direct counts of bacteria from the caecal contents of wild koalas and found average counts of 3.9×10^{10} per g wet matter. However, many of these (in particular gram negative cocci) were in close association with fibrous plant particles and so these counts are likely to be an underestimate of total numbers.

Recently, anaerobic fungi have been recognized as important colonizers of fibrous particles in digesta from several herbivores including domestic and wild ruminants, macropod marsupials, horses and elephants (Bauchop, 1979, 1980), although they are absent from the caecal digesta of Brushtail Possums and Rabbits in New Zealand. Although some eucalypts contain antifungal compounds (Egawa *et al.*, 1977) factors other than fibre appear to influence fungal colonization (e.g. dietary sulphur: Akin *et al.*, 1983) and more information is needed on fungal ecology before their occurrence can be predicted. The absence of flagellate protozoa in the hindgut of either species may possibly reflect a low proportion of soluble carbohydrates reaching the hindgut. While protozoa have not been observed in the hindgut of Koalas (London, 1981), or Brushtail Possums fed a high fibre artificial diet (Fitzgerald *et al.*, 1980), Clarke (unpub. in Fitzgerald *et al.*, 1981) has observed them in the caecum of wild Brushtail Possums in New Zealand.

The present observations showed that bacterial attachment occurred only on the broken surfaces of plant fragments. While few bacteria were seen on the undamaged surface of the epidermis of either eucalypt, large numbers of rods and cocci were observed on the mesophyll and epidermal cell walls. Similarly, few bacteria were seen on the highly lignified vessel elements. Clearly, the greater the available broken surface area, the greater the extent of bacterial attachment and consequent digestibility. Many authors (Akin, 1976, Akin and Amos, 1975, Dinsdale *et al.*, 1978) have stresed the importance of this effect. Bauchop (1980) found that all major bacterial (as well as fungal and protozoal) attachment in the rumen of sheep occurred at sites of physical damage or minor lesions on the plant.

The degraded areas surrounding many of the bacteria appeared similar to those seen in particles incubated in rumen fluid (Akin and Barton, 1983, Dinsdale *et al.*, 1978). The uniform shape of these zones tends to confirm that they have been formed by bacterial action. Some of these bacteria appeared to be attached to the plant surface by extracellular fibrous material. While the mechanisms by which bacteria attach to surfaces are not well understood (Costerton *et al.*, 1978) similar fibrous structures have been implicated in the attachment of rumen bacteria to plant tissues. Transmission electron micrographs (TEM) have shown that rumen bacteria can degrade mesophyll and parenchyma bundle sheath tissues without attachment (Akin and Amos, 1975, Akin and Barton, 1983). Extracellular enzymes have been reported in several species of cellulolytic micro-organisms. For example, Smith *et al.* (1973) showed that extracellular enzymes from *Ruminococcus albus* digested up to 65% of ground or blended cellulose. Akin (1979) concluded that attachment by micro-organisms was required for degradation of the more resistant tissues. Although many bacteria in the present study did not appear to be attached to plant tissues by extracellular material, TEM studies would be needed to determine whether degradation occurred by extracellular enzymes.

In conclusion, the pattern of tissue degradation and bacterial attachment in the hindgut of these two species appears similar to that found in the rumen. While there appeared to be few qualitative differences between the two species, the finer caecal particle size found in the Greater Glider probably allowed greater bacterial attachment than that found in the Brushtail Possum.

4.11 **Digestion of eucalypt foliage by arboreal marsupials**

The digestibility of *Eucalyptus* foliage has now been investigated in four species of arboreal marsupials which use eucalypt foliage as a food source to varying extents. These are the Koala (Cork *et al.*, 1983) Ringtail Possum (Chilcott and Hume, 1984a) and the two species examined here, the Greater Glider and the Brushtail Possum. The digestibilities of all fibre components by the four species are summarized and compared in

Table 4.9. It is generally accepted that larger herbivores should be able to utilize fibrous foods more readily than smaller animals (Janis, 1976, Hume and Warner, 1980, Van Soest, 1982). This is because smaller animals have higher mass-specific energy requirements than larger animals (Parra, 1978) and smaller absolute gut volumes. However, the data in Table 4.9 show almost completely the opposite trend with the largest animal, the Koala having the lowest total cell wall digestibility and the smallest animal, the Ringtail Possum appearing to be the most efficient. Recently however, Uden and Van Soest (1982) showed that although fibre digestibility increased with increasing body size in ruminants, the opposite was true in equines and rabbits. However, while all animals in Uden and Van Soest's (1982) study were fed the same fibre source, differences in dietary form (e.g. the rabbits were given ground and pelleted hay) probably confound these results.

It is necessary to examine a number of both plant- and animal-related factors that could have been influential in the above trend (Table 4.9). The first point to consider is the effect of dietary differences. All four species have been maintained on the foliage of different eucalypt species - the Koala on *E. punctata*, the Ringtail Possum on *E. andrewsii ssp campanulata*, the Greater Glider on *E. radiata ssp radiata* and the Brushtail Possum on *E. melliodora*. *Eucalyptus* is a variable genus (Pryor, 1976), and although the four species had similar cell wall and lignin contents it is possible that differences in lignin structure or the relationship of the lignin to the cellulose was responsible for the differences in digestibility. Little is known of the structure of lignins in most leaf tissues, especially *Eucalyptus*. However, the differences in the UV spectra (at 280nm) of the two species used in this study suggest differences in the content of true lignins but similar contents of phenolic acids (McCampbell and Thomas, 1972, Morrison, 1980). The biological significance of these spectral differences cannot yet be ascertained, but they do suggest that variations in foliage lignins may be important in explaining variations in digestibility.

Allied to variations in lignin structure are variations in the polyphenolic content of the four eucalypt species (Hillis, 1966). The

tannin fraction in particular varies both qualitatively and quantitatively between eucalypts (Fox and MacCauley, 1977, MacCauley and Fox, 1980). For example, the *E. punctata* foliage used by Cork (1981) contained no condensed tannins although leaf extracts did precipitate proteins. In contrast, condensed tannins appear to be a major component of the total phenols of both *E. radiata* and *E. melliodora* foliage (Chapter 6). Although the inhibition of bacterial cellulases by plant tannins *in vitro* has often been demonstrated (Smart *et al.*, 1961) it has only recently been shown that tannins can affect fibre digestion *in vivo* (Barry and Duncan, 1984, Barry and Manley, 1984 and Chapter 6). The data to be discussed in Chapter 6 suggest that *E. melliodora* tannins had significant effects on the level of total cell wall digestibility by Brushtail Possums. The consequences of this will be further discussed in that chapter.

Of the four species of marsupial folivore, only the Ringtail Possum is coprophagous. The Koala produces caecotrophic material during weaning of the young, but this is never ingested by adult animals (Minchin, 1937). The Ringtail Possum has been shown to ingest caecotrophes during the light phase of the day (Chilcott and Hume, 1985). This material has a lower NDF and ADF content than the "hard faeces" although the lignin content of the two faecal types is similar. In contrast, Uden and Van Soest (1982) showed that the lignin content of rabbit caecotrophes was only half that of the "hard faeces". Nevertheless, reingestion of this material should allow a second period of fermentation with a resultant increase in fibre digestibility. Although Chilcott (1982) did not consider the effects caecotrophy could have on fibre digestibility in the Ringtail Possum, Stephens (1977) has shown that prevention of caecotrophy in growing domestic rabbits fed high quality diets had no effect on the digestibility of ADF. On the other hand Pehrson (1983a) showed that prevention of caecotrophy in Mountain Hares (*Lepus timidus*) led to a significant reduction in the digestibility of the ADF of blueberry shoots. While other authors have not specifically considered the fibre fraction in their studies, all have demonstrated an increased dry matter digestibility when caecotrophy was allowed (e.g. Hintz, 1969, Bailey, 1969, Thacker and Brandt, 1955). While the high lignin content of the soft faeces of the Ringtail Possum could be expected to have a deleterious effect on any

further fermentation, it is likely that part of the reason for the high fibre digestibility in this species is the practice of caecotrophy.

A second animal-related factor which has considerable bearing on the efficiency of fibre digestion is the degree of initial mastication. This effect could be expected to be greater in a hindgut fermenter than in a ruminant (see for example, Beever *et al.*, 1981). A greater available particulate surface area should result in more extensive microbial attachment and cell wall digestion. Gipps (1980) has compared the dental morphology of the Brushtail Possum (and Mountain Possum) with that found in the Greater Glider and Ringtail Possum. She concluded that the dental structure of the two petaurids was better suited to fine comminution of food by a cutting action together with controlled compression and shearing stresses. On the other hand the phalangerids depend on a coarse grinding and ripping action. This results in a finer initial particle size in the stomach of the Greater Glider and Ringtail Possum than in the Brushtail Possum. This conclusion is supported by the present study (Chapter 3). There appear to be few differences between the Greater Glider and Ringtail Possum (Chilcott and Hume 1985) in stomach particle size although the data for Ringtail Possums are confounded by the reingestion of fine caecal particles during caecotrophy. These patterns are consistent with the trend in fibre digestibility shown in Table 4.9.

4.12 **Summary**

There appeared to be little seasonal variation in foliage constituents in either *E. radiata* or *E. melliodora* and both foliages contained large proportions of lignified fibre. The digestibility of this fibre, although low, was similar to or higher than the digestibility of the fibre of foliage or browse diets fed to other herbivores. Scanning electron micrographs showed that a variety of bacteria degraded fibrous tissues by attaching to the cell wall. However, few bacteria were observed on the more highly lignified tissues.

Chapter 5

NITROGEN AND UREA METABOLISM IN THE GREATER GLIDER AND BRUSHTAIL POSSUM

5.1 Introduction

Tree foliage is generally low in nitrogen content and several authors have speculated on the ability of small folivores to meet their protein requirements (Hladik, 1978, Milton, 1979). Eucalypt foliage in particular is very low in nitrogen (Ullrey *et al.*, 1981, Cork, 1984) and while little is known of the biological value of this nitrogen, there exist several factors which may reduce its availability to folivores such as the Greater Glider and Brushtail Possum. These include the presence of tannins (Fox and MacCauley, 1977, MacCauley and Fox, 1980) and the possibility that nitrogenous compounds are needed for the detoxification of allelochemicals (Bolliger and Whitten, 1940, Martin, 1973).

Although the Brushtail Possum has been shown to have a low maintenance nitrogen requirement when fed semi-purified diets (Wellard and Hume, 1981a), nothing is known of this species' ability to maintain nitrogen balance on foliage diets. The Koala has a low maintenance nitrogen requirement when fed *E. punctata* foliage (Cork, 1981), but this may be a reflection of its low basal metabolic rate (Degabriele and Dawson, 1979). Recently, Degabriele (1981, 1983) has surmised that the abundance of the Koala is limited by a shortage of high nitrogen content food. Braithwaite *et al.* (1983) showed that high levels of foliage nutrients (particularly nitrogen, phosphorus and potassium) were highly correlated with the density of Greater Gliders in hardwood forests in southern N.S.W.

If nitrogen is such a limiting nutrient for eucalypt-feeding marsupials, they could be expected to possess adaptations for conserving nitrogen. In particular, the recycling of endogenously produced urea to the gut is likely to be important. This recycled nitrogen may benefit

microbial growth or, if reabsorbed, could increase nitrogen retention by the animal.

In this chapter, nitrogen intake and excretion were determined in conjunction with the feeding experiments described in Chapter 4. From these data, maintenance nitrogen requirements have been calculated for each species. Examination of these results showed that urinary nitrogen losses in the Greater Glider were more than half the nitrogen intake. The kinetics of urea metabolism in the two species was therefore examined using [^{14}C] urea as a tracer. These results in turn suggested that in Greater Gliders, urea was not the principal nitrogenous excretory product. Several experiments were then designed to examine both the composition of the urine of the Greater Glider and some of the factors influencing the types of nitrogenous products found in the urine.

5.2 **Materials and Methods**

5.2.1 **Nitrogen intake and retention**

Nitrogen intake and excretion were determined in both species in conjunction with the feeding experiments already described (Section 4.5). Unfortunately, one Brushtail Possum urinated through the front of the cage on the majority of days that urine was collected in two experiments (T2 and T3). This uncertainty led to the exclusion of these two observations from the calculations of nitrogen retention.

The total nitrogen content of the feed, feed residues, faeces and urine was determined as described in Section 2.4.4a. Non-dietary faecal nitrogen (NDFN: see Section 5.4) was determined as in Section 2.4.4b and from this, the truly digestible nitrogen intake was derived from the formula:

Truly Digestible Nitrogen Intake = Dietary N Intake - (Faecal N - NDFN)

Two regression techniques were also tested for their utility in predicting non-dietary faecal nitrogen (Hironaka *et al.*, 1970). The maintenance nitrogen requirement was determined as the intake of either dietary or truly digestible nitrogen at which nitrogen retention was zero.

5.2.2 **Urea metabolism**

The kinetics of [^{14}C] urea were determined in two periods involving five Greater Gliders and three Brushtail Possums per period. At the end of a seven-day measurement of nitrogen balance, each animal was injected intramuscularly (at about 1400 h) with about 3.7MBq [^{14}C] urea. The precise dose was determined by weighing the syringe before and after injection. The decline in specific radioactivity of [^{14}C] urea was followed in the urine, rather than in the plasma, since the low levels of plasma urea in both species would have necessitated the collection of very large blood samples. The validity of this procedure has been discussed by Regoeczi *et al.* (1965) in rabbits, by Dellow (1979) in macropods and by Cork (1981) in Koalas. Since bladder catheterization was not feasible in either species, the collection of samples depended on the natural frequency of urination. This varied between four and eight times per day in the Brushtail Possum and between four and twelve times per day in the Greater Glider.

Urine collection bottles were checked every 30 minutes for 48 h. If urine was present, the volume was measured and it was acidified (pH2-3) and stored at -10° C and the collection apparatus replaced with a clean unit. Urine volumes were corrected for the volume lost on the collection trays and the time of urination was taken as the midpoint between collections.

Prior to assay of radioactivity, CO_2 was bubbled through the acidified urines to ensure that none of the [^{14}C] label was present in bicarbonate. Preliminary experiments showed that less than 0.1% of added [^{14}C] bicarbonate remained in urine after this treatment. Diluted (1:10) urine was counted in scintillation fluid. Samples and the injection solution were corrected for quenching by the automatic external standards

TABLE 5.1a: *Intake, excretion, retention and apparent digestibility of nitrogen in Greater Gliders (values expressed as* $g.kgW^{-0.75}.d^{-1}$*) (Mean ± SE)*

Experiment	Nitrogen intake	Faecal nitrogen	Urinary nitrogen	Nitrogen balance	Apparent digestibility of nitrogen (%)
P1	0.78 ± 0.02^{bc}	0.34 ± 0.01^{b}	0.35 ± 0.01^{a}	$+0.09 \pm 0.02^{ad}$	56.5 ± 1.9^{a}
P2	0.70 ± 0.01^{cd}	$0.37 \pm 0,01^{a}$	0.37 ± 0.03^{a}	-0.04 ± 0.01^{c}	47.7 ± 1.3^{c}
P3	0.92 ± 0.03^{a}	0.39 ± 0.02^{a}	0.39 ± 0.03^{a}	$+0.14 \pm 0.02^{ab}$	57.4 ± 1.7^{a}
P4	0.91 ± 0.03^{a}	0.39 ± 0.01^{a}	0.35 ± 0.02^{a}	$+0.17 \pm 0.03^{bc}$	57.5 ± 1.6^{a}
P5	0.84 ± 0.03^{b}	0.35 ± 0.01^{ab}	0.42 ± 0.02^{a}	$+0.08 \pm 0.04^{d}$	58.4 ± 2.0^{a}
P6	0.88 ± 0.02^{a}	0.37 ± 0.01^{a}	0.42 ± 0.03^{a}	$+0.10 \pm 0.03^{a}$	57.8 ± 1.0^{a}
P7	1.04 ± 0.05^{e}	0.35 ± 0.02^{b}	0.49 ± 0.03^{b}	$+0.20 \pm 0.02^{c}$	66.1 ± 1.6^{b}
P8	0.66 ± 0.02^{d}	0.30 ± 0.01^{c}	0.40 ± 0.02^{a}	-0.04 ± 0.03^{e}	54.6 ± 0.7^{a}
Mean	0.84 ± 0.02	0.36 ± 0.01	0.40 ± 0.01	+0.09 ± 0.01	57.0 ± 0.9
Range	0.59-1.19	0.26-0.44	0.27-0.57	-0.13-0.26	43.0-70.5

a,b,c,d,e = statistical analysis code within columns ($P < 0.05$)

TABLE 5.1b: *Intake, excretion, retention and apparent digestibility of nitrogen in Brushtail Possums (values expressed as* $g.kgW^{-0.75}.d^{-1}$*) (Mean ± SE)*

Experiment	Nitrogen intake	Faecal nitrogen	Urinary nitrogen	Nitrogen balance	Apparent digestibility of nitrogen (%)
T1	0.64 ± 0.03^{a}	0.40 ± 0.02^{a}	0.16 ± 0.01^{a}	$+0.07 \pm 0.02^{a}$	36.1 ± 2.5^{a}
T2	0.63 ± 0.01^{a}	0.44 ± 0.04^{a}	0.13 ± 0.00^{a}	$+0.07 \pm 0.01^{a}$	31.0 ± 2.9^{a}
T3	0.57 ± 0.02^{a}	0.38 ± 0.00^{a}	0.19 ± 0.02^{a}	$+0.01 \pm 0.04^{a}$	33.9 ± 3.4^{a}
T4	0.50 ± 0.03^{b}	0.35 ± 0.07^{a}	0.17 ± 0.02^{a}	-0.02 ± 0.01^{a}	29.4 ± 7.5^{a}
T5	0.54 ± 0.01^{ab}	0.36 ± 0.03^{a}	0.17 ± 0.04^{a}	$+0.00 \pm 0.03^{a}$	32.4 ± 3.9^{a}
Mean	0.58 ± 0.02	0.39 ± 0.02	0.16 ± 0.01	+0.03 ± 0.01	33.2 ± 1.8
Range	0.40-0.78	0.28-0.51	0.11-0.24	-0.05-0.14	15.6-43.3

a,b = statistical analysis code within columns ($P < 0.05$)

method. The urea content of each sample was determined as outlined in Section 2.4.4c. Urea kinetic parameters were computed using the procedures described by Cocimano and Leng (1967), Robbins *et al.* (1974) and Emmanuel *et al.* (1976).

5.2.3 **Composition of urinary nitrogen**

Urine samples were collected directly from the cloaca of five Greater Gliders and four Brushtail Possums feeding on *E. radiata* and *E. melliodora* foliage respectively. The urine was frozen immediately in liquid nitrogen. These samples were analysed for total nitrogen, urea, creatinine, allantoin and uric acid as described in Section 2.4.4. Blood samples were collected at the same time from the Greater Gliders (as described in Section 10.2.2) and by cardiac puncture from the Brushtail Possums. The plasma was separated by centrifugation and analysed for urea content.

5.2.4 **Alteration of Greater Glider urinary urea:ammonia ratio**

Six Greater Gliders were randomly allocated to two groups. One group was offered *E. radiata* foliage while the other was offered *Angophora floribunda* foliage. The animals would only eat the young *Angophora* leaves and since the experiment was conducted at the end of the growing season (March, 1983), acceptable leaves were in short supply. All animals' drinking water was replaced with 60 ml of a 10% glucose solution to ensure that any increase in urinary urea excretion observed was not due to body protein catabolism. Discrete urine samples were collected daily at 1600 h for three days. The treatments were then reversed and urine collected for a further three days.

5.3 **Results**

5.3.1 **Nitrogen intake and excretion**

Tables 5.1a and 5.1b give details of the intake, excretion and

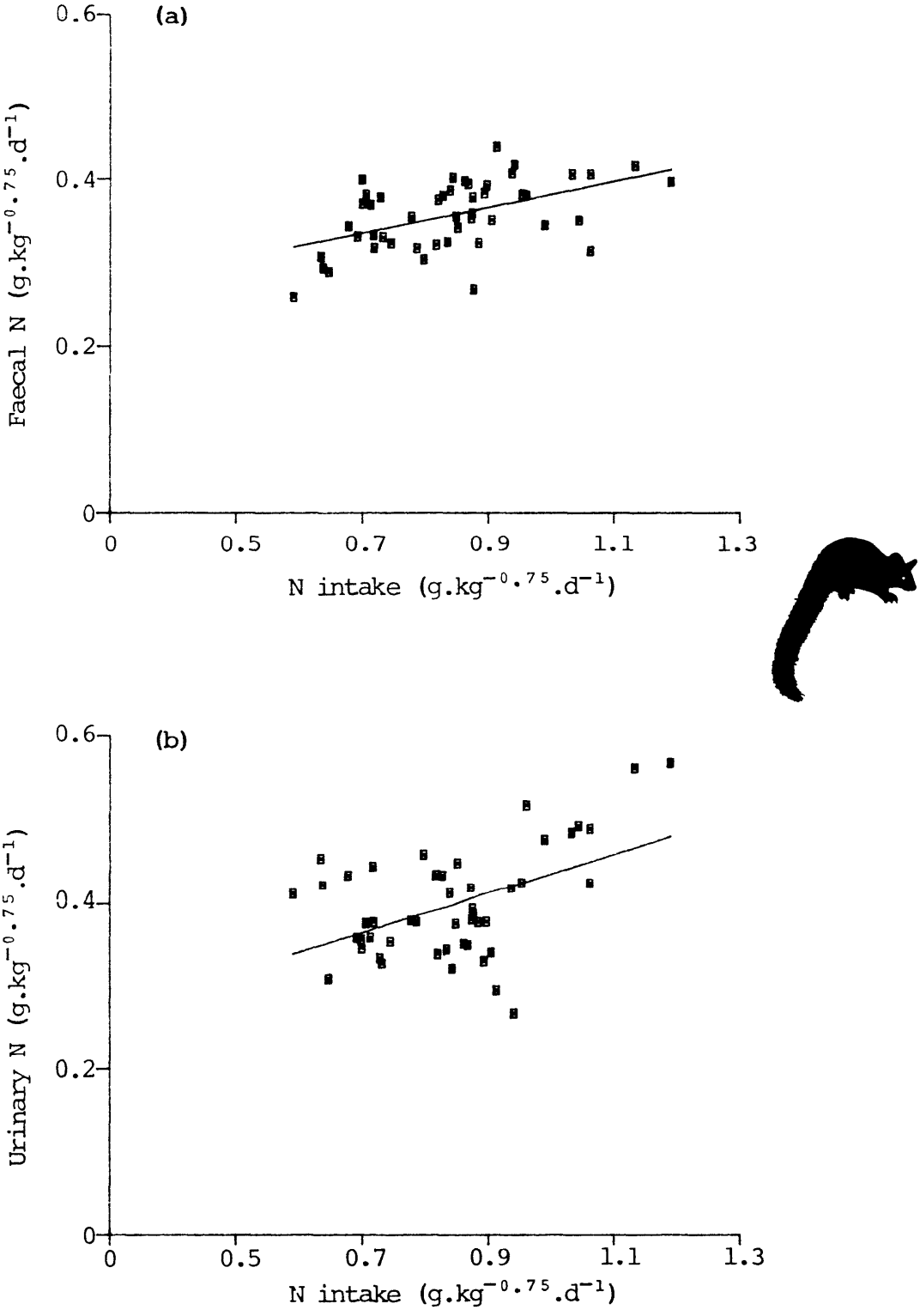

FIGURE 5.1: *Relationship between nitrogen intake by Greater Gliders and -*
(a) faecal nitrogen excretion; and
(b) urinary nitrogen excretion.

Regression equations:
(a) y = 0.226 + 0.155x, r = 0.491 (P<0.001), RSD = 0.36
(b) y = 0.200 + 0.236x, r = 0.470 (P<0.001), RSD = 0.058

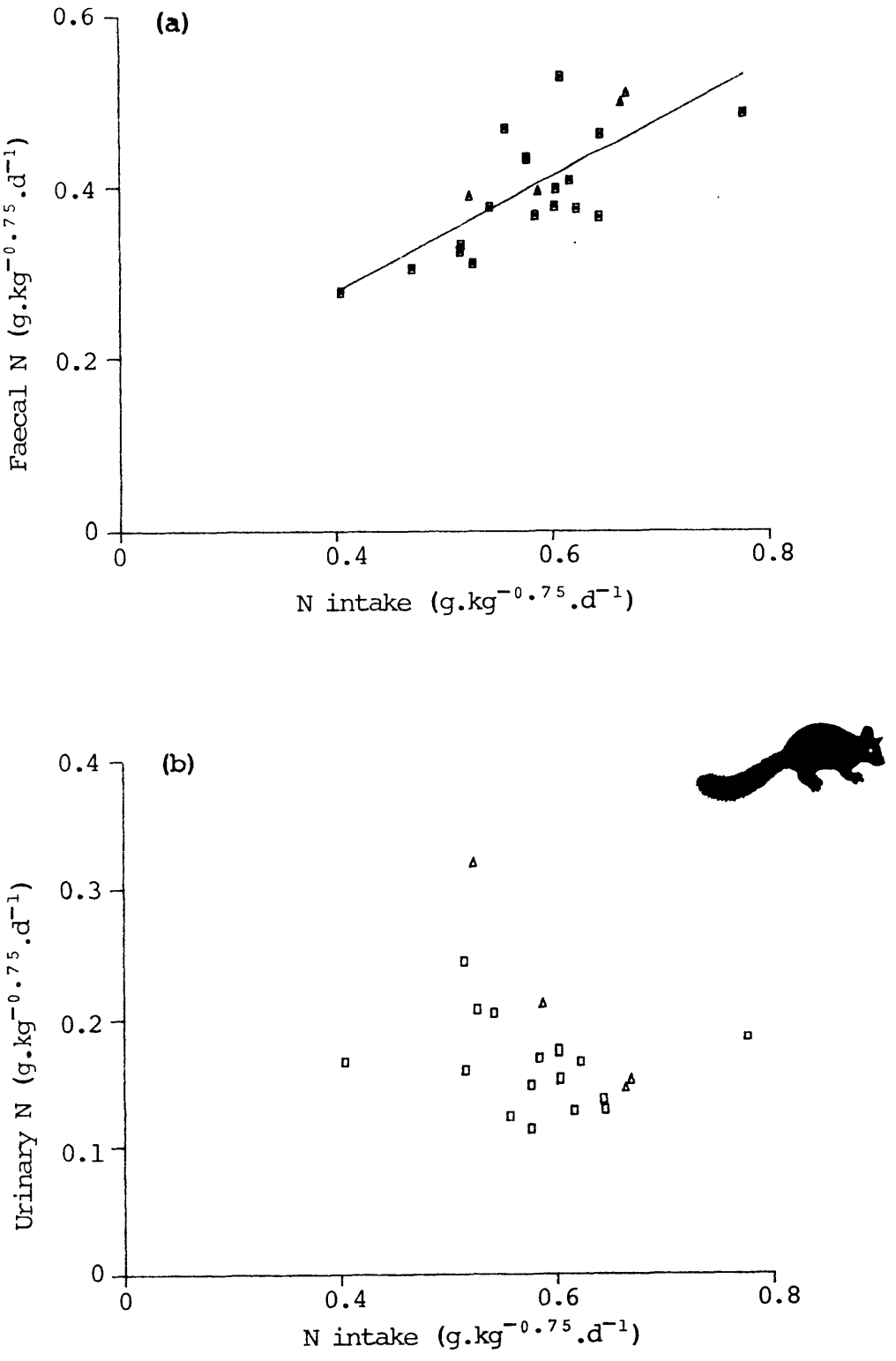

FIGURE 5.2: *Relationship between nitrogen intake by Brushtail Possums and -*
(a) faecal nitrogen excretion; and
(b) urinary nitrogen excretion.

Regression equations:
(a) y = 0.007 + 0.676x, r = 0.725 (P<0.001), RSD = 0.490
(b) relationship not significant

apparent digestibility of nitrogen in Greater Gliders and Brushtail Possums respectively. *E. radiata* nitrogen content varied from 1.6-2.4% N and dietary nitrogen intakes by Greater Gliders ranged from 0.59-1.19 $gN \cdot kgW^{-0.75} \cdot d^{-1}$. While *E. melliodora* foliage had a lower nitrogen content (1.3-1.7% N), the lower nitrogen intakes of the Brushtail Possums (0.40-0.78 g $N \cdot kgW^{-0.75} \cdot d^{-1}$) were principally a reflection of their lower dry matter intakes.

The major source of nitrogen loss in the Greater Gliders was via the urine. The urinary nitrogen excretion rate was 0.40 g $N \cdot kgW^{-0.75} \cdot d^{-1}$ which was 48% of the dietary nitrogen intake. Faecal nitrogen losses averaged 0.36 g $N \cdot kgW^{-0.75} \cdot d^{-1}$, 42% of the dietary nitrogen intake. There were significant ($P<0.001$) relationships between dietary nitrogen intake and both faecal (Figure 5.1a) and urinary (Figure 5.1b) nitrogen excretion. Extrapolation of the relationship between urinary nitrogen excretion and dietary nitrogen intake gave a Y intercept value of 0.2 g $N \cdot kgW^{-0.75} \cdot d^{-1}$ at zero intake. However, this is unlikely to be a reliable estimate of the endogenous urinary nitrogen excretion of Greater Gliders since the confidence interval of the regression at zero nitrogen intake was wide.

The mean nitrogen balance in the Greater Gliders (0.09 g $N \cdot kgW^{-0.75} \cdot d^{-1}$) was positive although negative balances were recorded in Experiments P2 and P8. The nitrogen content of the *E. radiata* leaf used in Experiment P2 was the lowest encountered during the study, but the negative nitrogen balances of Experiment P8 are probably attributable to the low dry matter intakes in this experiment. After adjusting for the effect of nitrogen intake, there were no significant differences in nitrogen excretion or retention between summer and winter experiments.

In contrast, in the Brushtail Possums, faecal nitrogen excretion was the major source of nitrogen loss (0.39 g $N \cdot kgW^{-0.75} \cdot d^{-1}$) (67% of nitrogen intake); urinary nitrogen excretion (0.16 g $N \cdot kgW^{-0.75} \cdot d^{-1}$) represented only 28% of the dietary nitrogen intake. There was a significant ($P<0.001$) relationship between faecal nitrogen output and dietary nitrogen intake (Figure 5.2a). There was no relationship between urinary nitrogen excretion and nitrogen intake (Figure 5.2b). This can

TABLE 5.2a: *Non-dietary faecal nitrogen (NDFN) excretion and the true digestibility of dietary nitrogen in Greater Gliders (Mean ± SE)*

Experiment	Non-dietary faecal nitrogen			True digestibility of nitrogen (%)	Truly digestible nitrogen intake ($g.kgW^{-0.75}.d^{-1}$)
	$g.kgW^{-0.75}.d^{-1}$	g.kg DMI	g.kg DMO		
P1	0.23 ± 0.01^{a}	4.8 ± 0.3^{b}	11.3 ± 0.6^{b}	86.0 ± 0.9^{a}	0.67 ± 0.02^{b}
P2	0.24 ± 0.01^{ab}	5.5 ± 0.1^{a}	11.9 ± 0.4^{ab}	82.7 ± 0.9^{c}	0.58 ± 0.01^{c}
P3	0.26 ± 0.01^{b}	5.7 ± 0.2^{a}	13.4 ± 0.5^{a}	85.5 ± 0.6^{ab}	0.79 ± 0.03^{a}
P4	0.26 ± 0.01^{b}	5.7 ± 0.2^{a}	13.6 ± 0.5^{ac}	85.8 ± 1.0^{a}	0.79 ± 0.03^{a}
P5	0.21 ± 0.01^{d}	4.9 ± 0.2^{b}	11.5 ± 0.3^{ab}	83.4 ± 1.4^{b}	0.70 ± 0.03^{b}
P6	0.22 ± 0.01^{ad}	4.8 ± 0.1^{b}	12.1 ± 0.3^{ab}	83.2 ± 0.8^{bc}	0.74 ± 0.02^{ab}
P7	0.24 ± 0.01^{a}	5.2 ± 0.2^{ab}	14.8 ± 0.9^{c}	89.2 ± 0.8^{a}	0.93 ± 0.04^{a}
P8	0.11 ± 0.01^{c}	3.3 ± 0.2^{c}	8.0 ± 0.5^{d}	71.8 ± 1.2^{d}	0.47 ± 0.02^{d}
Mean	0.22 ± 0.01	5.0 ± 0.1	12.1 ± 0.3	83.5 ± 0.77	0.71 ± 0.02
Range	0.09-0.29	2.6-6.7	6.2-19.1	68.3-91.4	0.44-1.04

a,b,c,d = statistical analysis code within columns ($P < 0.05$)

TABLE 5.2b: *Non-dietary faecal nitrogen (NDFN) excretion and the true digestibility of dietary nitrogen in Brushtail Possums (Mean ± SE)*

Experiment	Non-dietary faecal nitrogen			True digestibility of nitrogen (%)	Truly digestible nitrogen intake ($g.kgW^{-0.75}.d^{-1}$)
	$g.kgW^{-0.75}.d^{-1}$	g.kg DMI	g.kg DMO		
T1	0.24 ± 0.01^{a}	6.1 ± 0.3^{b}	13.5 ± 0.9^{a}	74.8 ± 1.3^{b}	0.47 ± 0.02^{a}
T2	0.23 ± 0.01^{a}	6.1 ± 0.1^{ab}	12.3 ± 0.9^{a}	67.8 ± 2.2^{ab}	0.43 ± 0.01^{ab}
T3	0.30 ± 0.06^{a}	8.7 ± 0.1^{a}	17.3 ± 0.2^{a}	79.8 ± 0.8^{a}	0.43 ± 0.04^{ab}
T4	0.26 ± 0.05^{a}	8.6 ± 1.4^{a}	16.7 ± 2.2^{a}	81.2 ± 3.9^{a}	0.40 ± 0.02^{b}
T5	0.26 ± 0.04^{a}	8.1 ± 0.9^{a}	16.3 ± 1.6^{a}	81.1 ± 4.3^{a}	0.43 ± 0.03^{ab}
Mean	0.26 ± 0.01	7.4 ± 0.1	14.9 ± 0.1	77.4 ± 1.4	0.44 ± 0.00
Range	0.18-0.42	4.9-10.9	10.8-20.4	65.1-89.0	0.36-0.55

a,b = statistical analysis code within columns ($P < 0.05$)

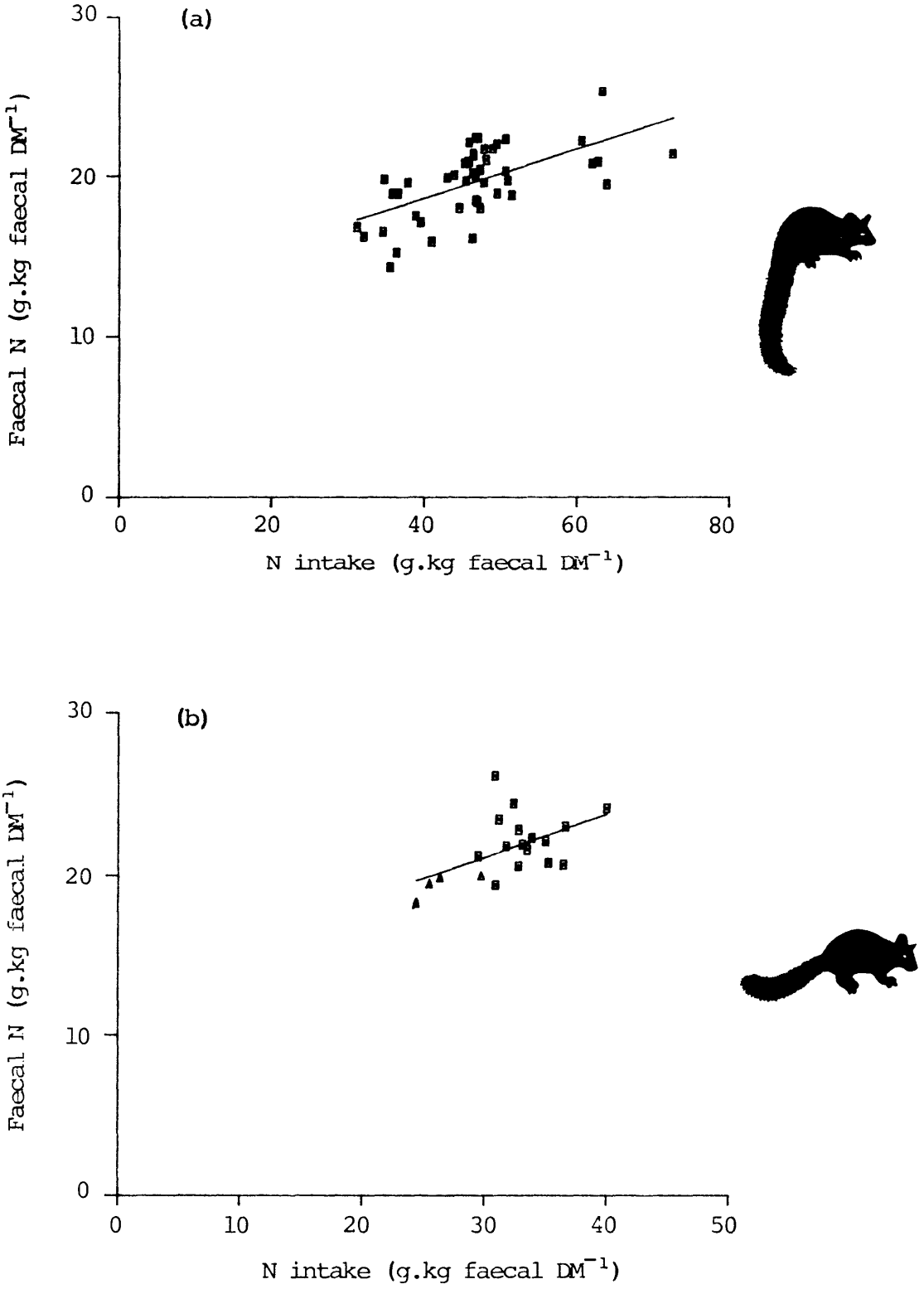

FIGURE 5.3: *Relationship between nitrogen intake and faecal nitrogen excretion in -*
(a) Greater Gliders; and
(b) Brushtail Possums.

Regression equations:
(a) y = 12.467 + 0.154x, r = 0.590 (P<0.001), RSD = 1.781
(b) y = 13.263 + 0.262x, r = 0.483 (P<0.05), RSD = 1.687

TABLE 5.3: *Summary of techniques used to estimate NDFN excretion in the Greater Glider and Brushtail Possum*

	Technique	Greater Glider	Brushtail Possum[3]
1	Regression of Dietary N (g/kg faecal DMO[1]) v. Faecal N (g/kg faecal DMO)	12.5 (9.6 - 15.4)[4]	13.3 (6.8 - 19.9)[4]
2	Regression of Dietary N (g/kg DMI[2]) v. apparently digestible N (g/kg DMI)	6.4 (4.3 - 8.6)[4]	6.0 (1.8 - 13.7)[4]
3	Mason (1969) - Neutral detergent soluble faecal nitrogen (g/kg DMO)	12.2	13.5
4	Mason (1969) - Neutral detergent soluble faecal nitrogen (g/kg DMI)	5.0	6.9

[1] Faecal dry matter excretion

[2] Dry matter intake

[3] Regressions 1 and 2 and means of 3 and 4 calculated including data of treatment 4, Table 6.5

[4] 95% confidence intervals for y-intercept of regressions in Figures 5.3 and 5.4

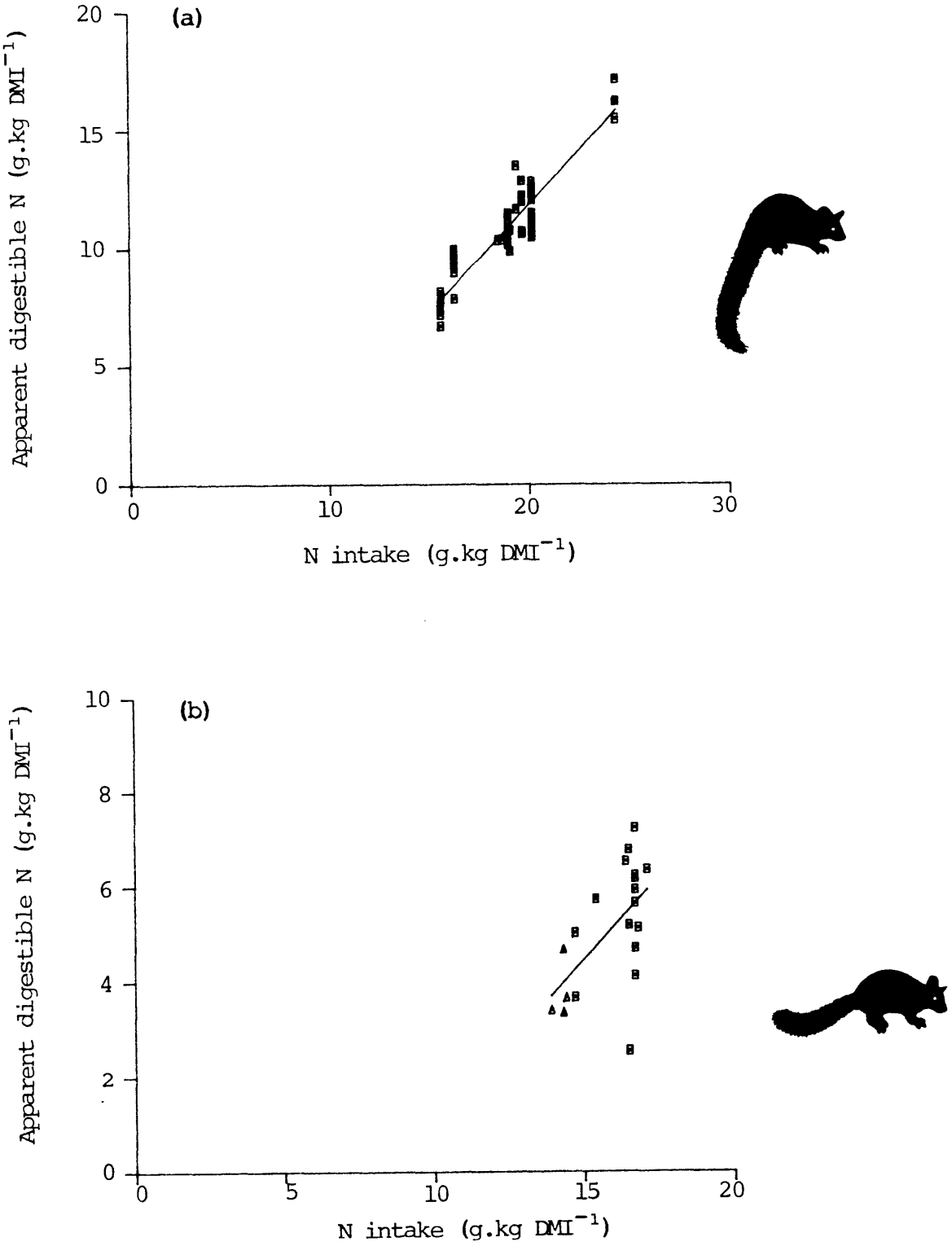

FIGURE 5.4: *Relationship between dietary nitrogen intake and apparently digestible nitrogen in -*
(a) Greater Gliders; and
(b) Brushtail Possums.

Regression equations:
(a) y = -6.415 + 0.911x, r = 0.923 (P<0.001), RSD = 0.843
(b) y = -5.986 + 0.697x, r = 0.548 (P<0.05), RSD = 1.094

probably be attributed to the narrow range of nitrogen intakes of Brushtail Possums on the *E. melliodora* diets. Nitrogen balance was close to equilibrium in all experiments although both positive and negative values were recorded.

5.3.2 **Non-dietary faecal nitrogen and the true digestibility of dietary nitrogen**

Details of NDFN excretion and the true digestibility of nitrogen in both species are given in Tables 5.2a and 5.2b. NDFN excretion in the Greater Glider, determined by the detergent method (Mason, 1969) was 0.22 g $N \cdot kgW^{-0.75} \cdot d^{-1}$, similar to the value in the Brushtail Possum (0.26 g $N \cdot kgW^{-0.75} \cdot d^{-1}$). However, the lower dry matter intakes and dry matter digestibilities of the Brushtail Possums meant that NDFN excretion in this species was much greater when expressed in terms of dry matter intake (DMI) or faecal dry matter output (DMO). Hence, in the Greater Gliders, NDFN was 5.0 g N·kg DMI or 12.1 g N·kg faecal DMO, whereas in the Brushtail Possums NDFN amounted to 7.4 g N·kg DMI and 14.9 g N·kg faecal DMO. Of the two regressions tested as predictors of NDFN excretion, one (Figure 5.3a and 5.3b) gave values that were almost identical with those estimated by the chemical partitioning in both species (Table 5.3). This was surprising since in both the Greater Gliders and the Brushtail Possums there were few observations at very low nitrogen intakes. Therefore, the confidence interval at zero nitrogen intake would have been wide. The second regression (Figure 5.4a and 5.4b) gave values which were different ($P<0.05$) from the chemical estimates in the Greater Gliders but similar in the Brushtail Possums. Again there were no observations near zero nitrogen intake.

5.3.3 **Maintenance nitrogen requirement**

There were significant relationships between nitrogen balance and dietary (Figure 5.5a) and truly digestible (Figure 5.5b) nitrogen intakes in the Greater Gliders. These relationships were best described by quadratic equations to account for the proportionally lower nitrogen

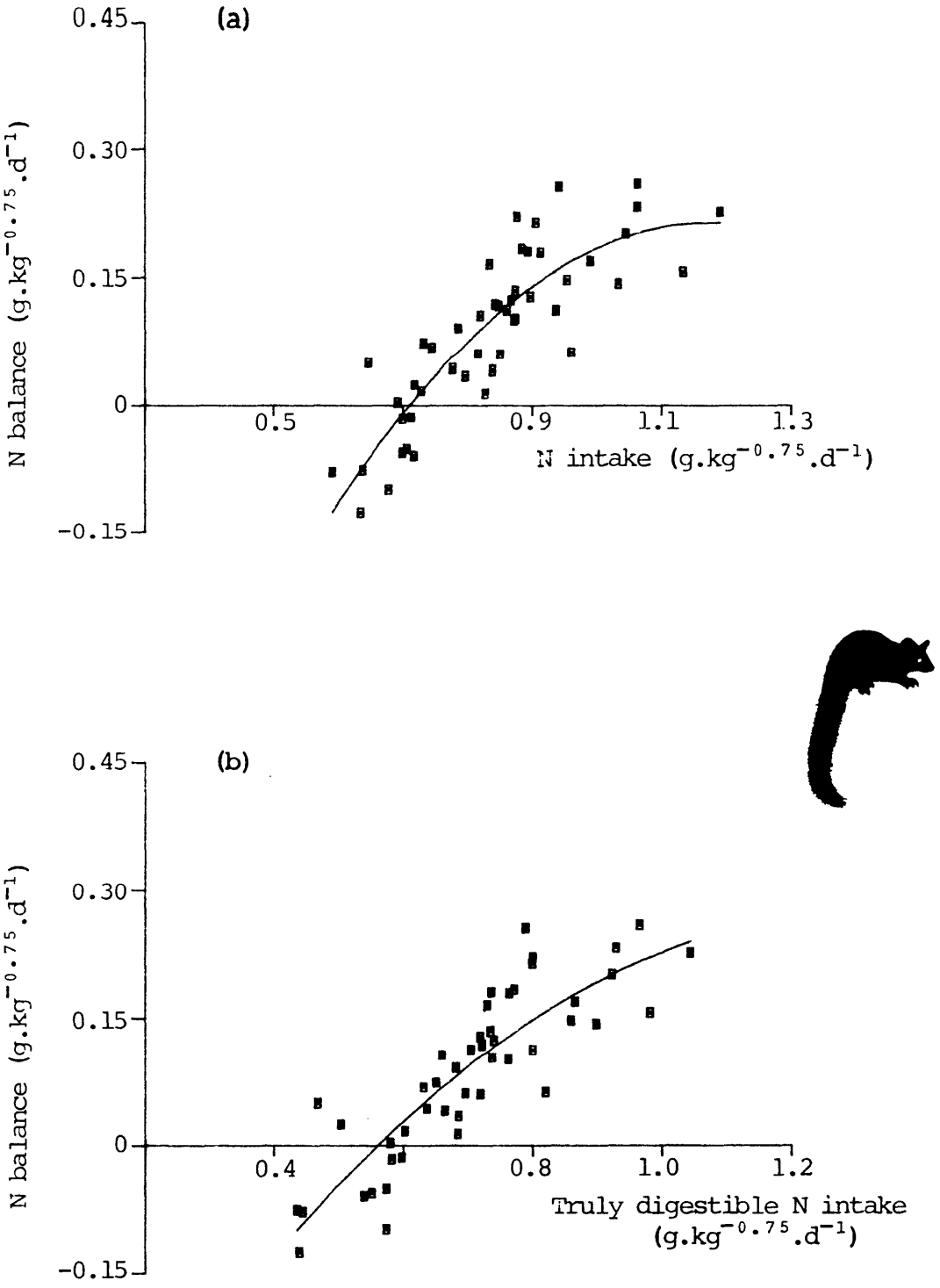

FIGURE 5.5: *Relationship between nitrogen balance in Greater Gliders and -*
(a) dietary nitrogen intake; and
(b) truly digestible nitrogen intake.

Regression equations:
(a) $y = -1.165 + 2.356x - 1.005x^2$ $r = 0.863$ ($P<0.001$) RSD = 0.050
(b) $y = -0.570 + 1.296x - 0.498x^2$ $r = 0.857$ ($P<0.001$) RSD = 0.051

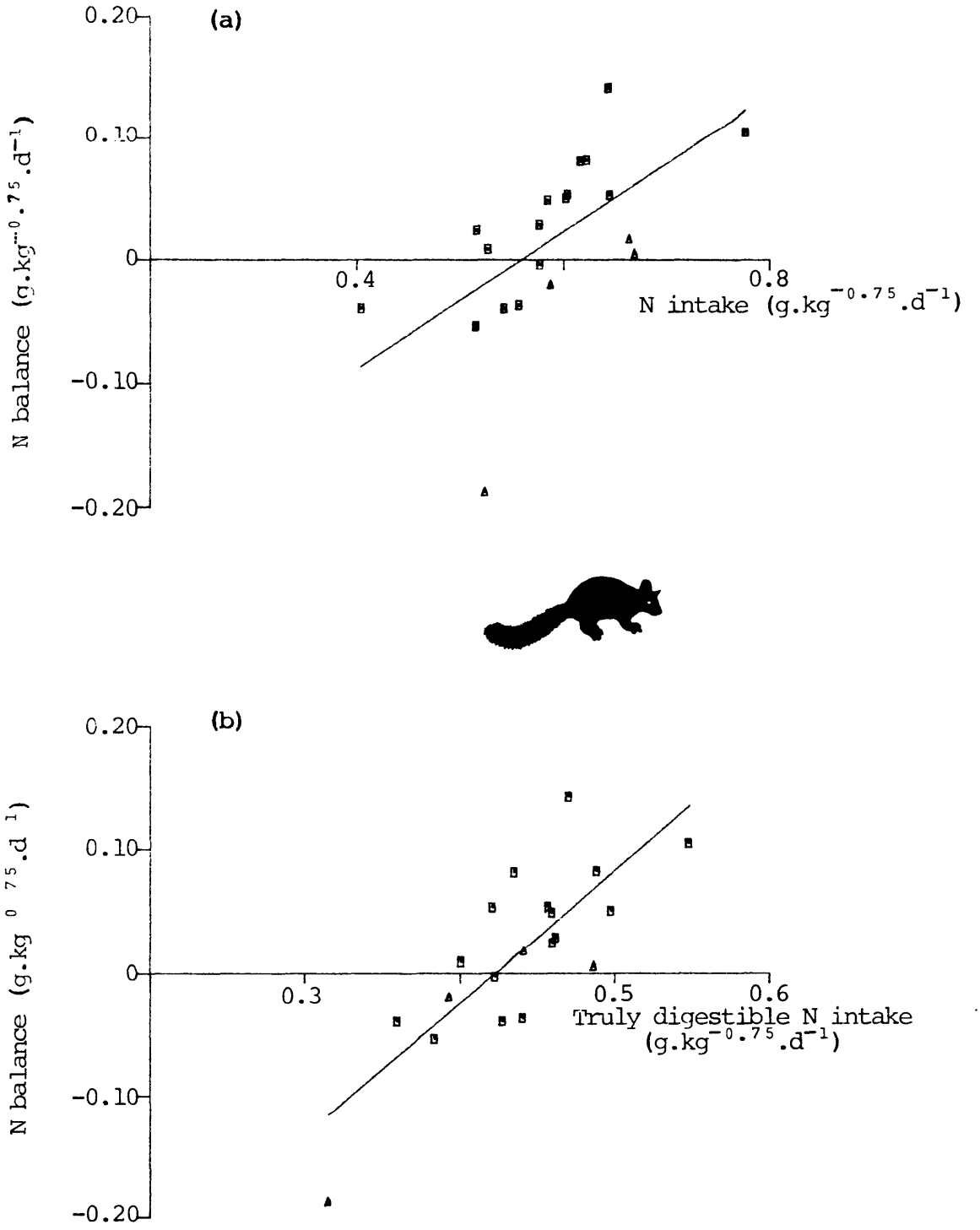

FIGURE 5.6: *Relationship between nitrogen balance in Brushtail Possums and -*
(a) dietary nitrogen intake; and
(b) truly digestible nitrogen intake.

Regression equations:
(a) $y = -0.312 + 0.558x$, $r = 0.578$ ($P<0.01$), RSD = 0.058
(b) $y = -0.452 + 1.071x$, $r = 0.780$ ($P<0.001$), RSD = 0.044

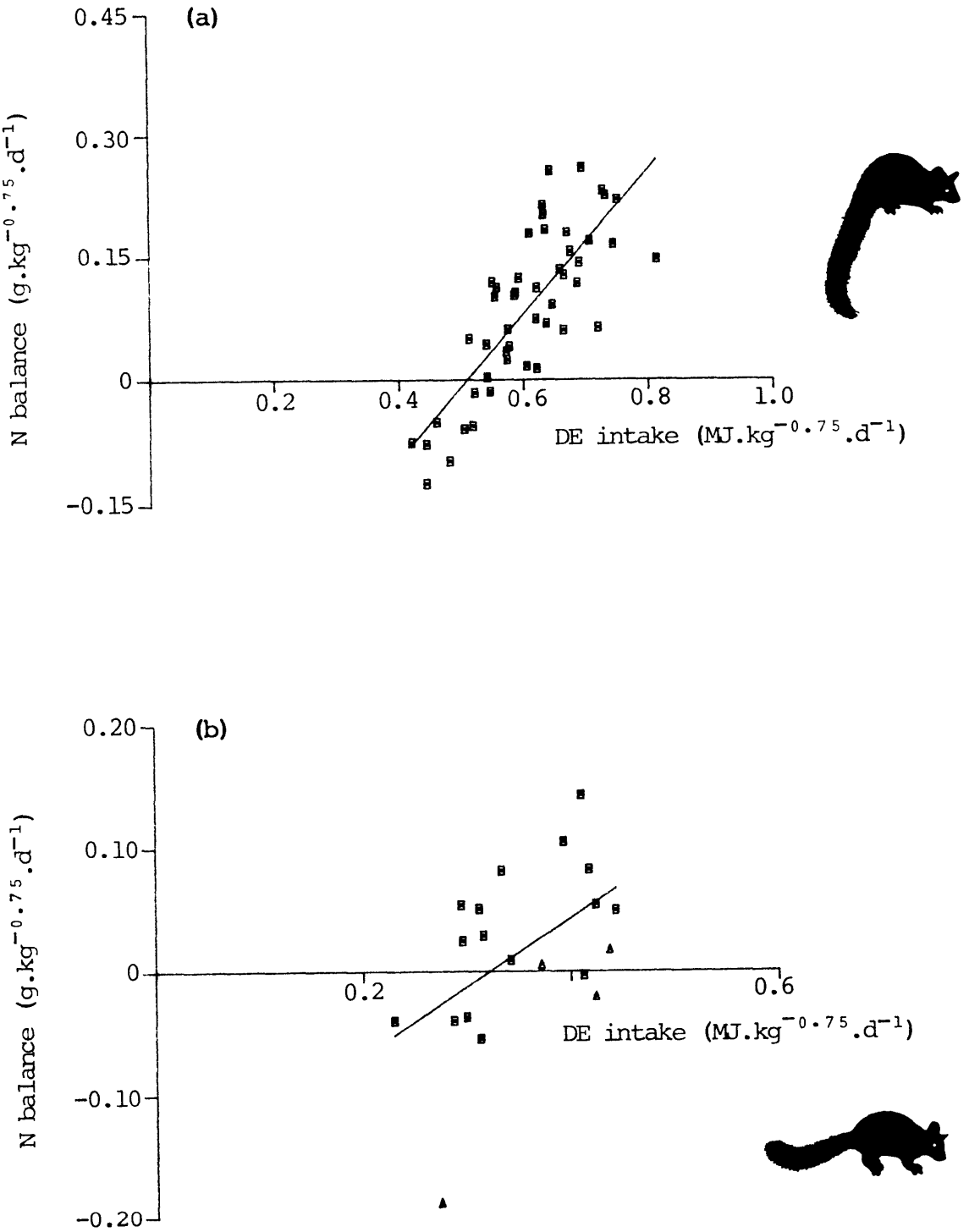

FIGURE 5.7: *Relationship between intake of digestible energy and nitrogen balance in (a) Greater Gliders; and (b) Brushtail Possums.*

Regression equations:
(a) $y = -0.450 + 0.882x$, $r = 0.792$ ($P<0.001$), RSD = 0.060
(b) $y = -0.177 + 0.550x$, $r = 0.460$ ($P<0.05$), RSD = 0.063

TABLE 5.4: *Kinetics of [^{14}C] urea in (a) Greater Gliders and (b) Brushtail Possums, derived using a single injection of [^{14}C] urea (Means ± SE)*

	Greater Gliders			Brushtail Possums		
	Expt 1	Expt 2	Mean[1]	Expt 1	Expt 2	Mean[2]
Body weight (kg)	1.139 ± 0.03	1.063 ± 0.05	1.101 ± 0.02	2.33 ± 0.16	2.59 ± 0.23	2.45 ± 0.10
Urea pool size ($mgN.kg^{-0.75}$)	19.3 ± 2.4[a]	16.9 ± 2.0[a]	18.1 ± 1.7	94.1 ± 11.7[c]	69.9 ± 6.4[d]	82.0 ± 6.5
Turnover time (min)	475 ± 56[a]	474 ± 43[a]	475 ± 33	812 ± 108[c]	741 ± 62[c]	777 ± 61
Urea entry rate ($mgN.kg^{-0.75}.d^{-1}$)	60.5 ± 5.3[a]	53.8 ± 7.0[a]	57.2 ± 4.2	168.0 ± 1.4	138.4 ± 16.4	153.2 ± 6.4
Urea excretion rate ($mgN.kg^{-0.75}.d^{-1}$)	2.9 ± 0.5[a]	4.3 ± 0.9[a]	3.6 ± 0.3	62.6 ± 12.5[c]	62.4 ± 19.4[d]	62.5 ± 11.4
Urea degradation rate ($mgN.kg^{-0.75}.d^{-1}$)	57.6 ± 5.5[a]	49.4 ± 6.5[a]	53.5 ± 3.8	105.7 ± 13.9[c]	76.8 ± 21.6[c]	91.3 ± 13.2
Urea degradation rate (% entry rate)	95.0 ± 1.1[a]	92.0 ± 1.3[b]	93.5 ± 0.8	62.6 ± 7.8[c]	55.3 ± 12.3[c]	59.0 ± 7.2

[1] n = 10

[2] n = 6

a,b = statistical analysis code within rows, Greater Gliders

c,d = statistical analysis code within rows, Brushtail Possums

retention at high nitrogen intakes. The maintenance nitrogen requirement was 0.70 g dietary $N \cdot kgW^{-0.75} \cdot d^{-1}$ or 0.56 g truly digestible $N \cdot kgW^{-0.75} \cdot d^{-1}$. In Brushtail Possums, the relationships between both dietary (Figure 5.6a) and truly digestible (Figure 5.6b) nitrogen intakes and nitrogen balance were best described by linear equations and the maintenance nitrogen requirement was 0.56 g dietary $N \cdot kgW^{-0.75} \cdot d^{-1}$ or 0.42 g truly digestible $N \cdot kgW^{-0.75} \cdot d^{-1}$. However, there was a significant relationship between digestible energy intake and nitrogen balance in both species (Figures 5.7a and 5.7b) which may be evidence that the animals were catabolizing some body tissues to meet essential tissue energy requirements and so the maintenance nitrogen requirements may be overestimates.

5.3.4 **Urea metabolism**

Details of the kinetics of [^{14}C] urea in both species are given in Table 5.4 The urea degradation rate (as a proportion of entry rate) was greater ($P<0.05$) in the first period in Greater Gliders but there were no other significant differences in any parameter of urea kinetics between periods. In Brushtail Possums, urea pool size and urea entry rate was higher ($P<0.05$) in the first period but there were no significant differences in other parameters. The urea entry rate was not related to either nitrogen intake, urea excretion rate or urea pool size in either species. Greater Gliders recycled 94% of the endogenously synthesised urea or 54 mg urea $N \cdot kgW^{-0.75} \cdot d^{-1}$. Brushtail Possums recycled 59% of synthesised urea or 90mg urea $N \cdot kgW^{-0.75} \cdot d^{-1}$.

5.3.5 **Composition of urinary nitrogen**

Table 5.5 shows the partitioning of the urinary nitrogen of both species between five compounds. About 89% of the total urinary nitrogen of the Greater Glider and 94% of the Brushtail Possum urinary nitrogen could be accounted for as urea, creatinine, ammonia, allantoin and uric acid. Ammonia was the dominant fraction in the Greater Glider urine but urea accounted for 53% of the urinary nitrogen of the Brushtail Possum. The

TABLE 5.5: *Composition of urinary nitrogen (% total N)*

Species	Urea-N	NH_4-N	Creatinine-N	Urate-N	Allantoin-N	Balance	Urea U/P ratio[1]
Greater Glider[2]	1.4	78.0	9.2	0.4	0.4	10.6	2.9
Brushtail Possum[3]	53.1	25.9	7.2	7.3	0.3	6.2	41.0

[1] Ratio of urea in urine to urea in plasma

[2] n = 5

[3] n = 4

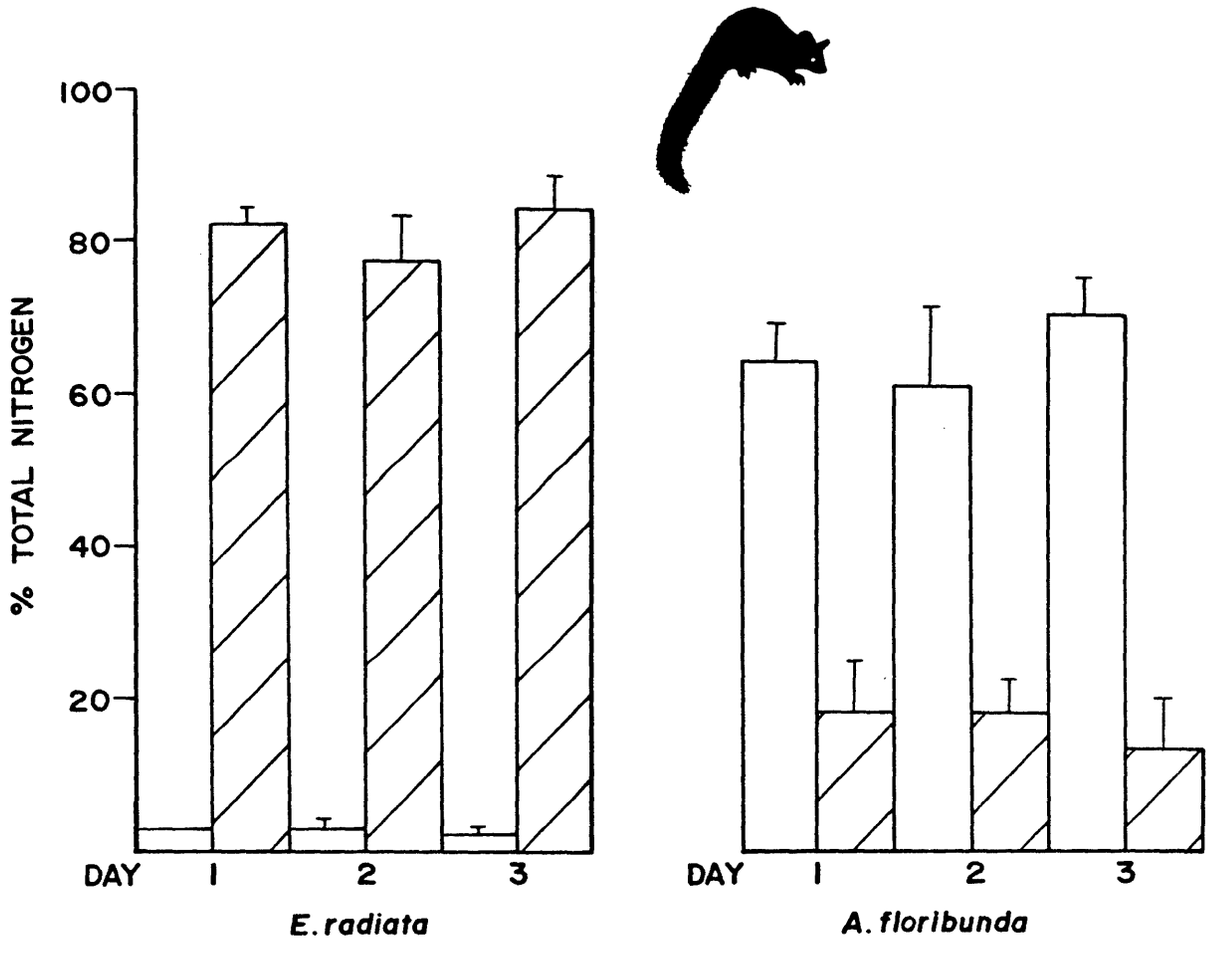

FIGURE 5.8: *Effect of changing the diet of Greater Gliders from* E. radiata *to* Angophora floribunda *on the proportion of urinary nitrogen excreted as urea (open bars) or* NH_4 *(shaded bars). Means ± SE.*

TABLE 5.6: *Maintenance nitrogen requirements of several marsupial and eutherian herbivores (all values* $gN.kg^{-0.75}.d^{-1}$)

	Dietary N requirement	Digestible N requirement	Reference
EUTHERIAN - Foregut fermenters			
Sheep	0.52	0.45	Moir and Williams (1950)
Caribou	-	0.46[1]	McEwan and Whitehead (1970)
White-tailed Deer	0.77	-	Holter *et al.* (1979)
- Hindgut fermenters			
Domestic rabbit	0.50	0.33[1,2]	Cork (1975)
Black-tailed Jackrabbit	0.69	0.45[1,2]	Nagy *et al.* (1976)
Mountain Hare	0.96	0.88[2]	Pehrson (1983b)
Rock Hyrax	0.31	0.21	Hume *et al.* (1980)
Horse	0.95	0.80	Slade and Robinson (1970)
MARSUPIAL - Foregut fermenters			
Eastern Grey Kangaroo	0.35	0.27	Foley *et al.* (1980)
Euro	0.36	0.15	Brown and Main (1967)
Red-necked Pademelon	0.60	0.53	Hume (1977b)
Tammar Wallaby	0.24	0.23	Hume (1977b)
- Hindgut fermenters			
Koala	0.28	0.27	Cork (1981)
Ringtail Possum	0.38	0.29[2] (0.62)[3]	Chilcott and Hume (1984b)
Greater Glider	0.70	0.56	Present study
Brushtail Possum (foliage)	0.56	0.42	Present study
Brushtail Possum (semi-purified)	0.20	0.19	Wellard and Hume (1981a)

[1] Apparently digestible N requirement - all other values, truly digestible N requirement

[2] Caecotrophy allowed

[3] Estimate in absence of caecotrophy

TABLE 5.7: *Partitioning of nitrogen excretion in several species of herbivorous marsupials at similar levels of truly digestible nitrogen intake (values expressed as* $gN.kg^{-0.75}.d^{-1}$)

Species	Diet	Truly digestible nitrogen intake	Faecal nitrogen excretion	Urinary nitrogen excretion	Non-dietary faecal nitrogen	Reference
FOREGUT FERMENTERS						
Tammar Wallaby	Chopped lucerne, hay and sugar	0.40 - 1.05	0.15 - 0.25	0.10 - 0.27	0.14	Hume 1977b
Red-necked Pademelon	Hay and sugar	0.40 - 1.20	0.10 - 0.43	0.25 - 0.59	n.a.	Hume 1977b
HINDGUT FERMENTERS						
Koala	Eucalypt foliage	0.38 - 0.58	0.21 - 0.36	0.05 - 0.14	0.24	Cork 1981
Ringtail Possum	Eucalypt foliage	0.28 - 0.48	0.15 - 0.26	0.15 - 0.27	0.10	Chilcott & Hume 1984b
Greater Glider	Eucalypt foliage	0.44 - 1.04	0.26 - 0.44	0.27 - 0.57	0.22	Present study
Brushtail Possum	Eucalypt foliage	0.36 - 0.55	0.28 - 0.51	0.11 - 0.24	0.26	Present study
Brushtail Possum	Semi-purified: HF	0.30 - 0.70	0.08 - 0.19	0.07 - 0.18	0.10	Wellard & Hume 1981a
Brushtail Possum	Semi-purified: LF	0.26 - 0.72	0.03 - 0.06	0.10 - 0.44	0.05	Wellard & Hume 1981a

HF = high fibre; LF = low fibre; n.a. = not available

urinary urea/plasma urea ratio was consistently greater than unity in both species.

5.3.6 **Greater Glider urinary nitrogen excretion**

Figure 5.8 shows the effect of feeding *Angophora floribunda* foliage on the ratio of urinary urea-N:ammonia-N in Greater Gliders. Animals eating *A. floribunda* excreted more nitrogen as urea ($P<0.001$) and less nitrogen as ammonia ($P<0.001$) than did animals fed *E. radiata* leaves. There were no significant differences in the ratio of urea-N:ammonia-N over the three days of the collection.

5.4 **Discussion**

The maintenance nitrogen requirements (MNR) of both the Greater Glider and the Brushtail Possum, when fed solely on eucalypt foliage, are greater than those recorded for all other marsupials except the Red-necked Pademelon. The values recorded here are within the range of values found for eutherian mammals. Table 5.6 shows the maintenance nitrogen requirements of a range of marsupial and eutherian species. The MNR of the Greater Glider is almost twice that of the closely related Ringtail Possum and of the Koala. The low requirement of the Ringtail Possum can be interpreted as a consequence of caecotrophy. Chilcott and Hume (1985) calculated that in the absence of caecotrophy the MNR of the Ringtail would be about 0.62g of truly digestible $N \cdot kgW^{-0.75} \cdot d^{-1}$, similar to the value reported here for the Greater Glider.

Table 5.7 compares the routes of nitrogen excretion in several species of marsupial at similar levels of truly digestible nitrogen intake. Clearly, the Red-necked Pademelon and the Greater Glider have a similar pattern of nitrogen loss with up to 50% of the truly digestible nitrogen intake being lost in the urine. Hume (1977b) suggested that the high MNR of the Red-necked Pademelon could be a reflection of a higher basal metabolic rate or of differences in the relationship between endogenous urinary nitrogen excretion and metabolic rate among marsupials. The low urinary nitrogen excretion of the Koala has been interpreted (Cork, 1981)

to be a consequence of the Koala's low basal metabolic rate (Degabriele and Dawson, 1979). This appears to be the principal reason for the low MNR of the Koala.

Faecal nitrogen losses are also important in explaining some of the differences in MNR between species. Faecal nitrogen losses in Brushtail possums were several times greater than those found by Wellard and Hume (1981a) at similar levels of nitrogen intake and dietary NDF. However, faecal nitrogen consists of nitrogen of endogenous origin as well as undigested dietary nitrogen. This endogenous nitrogen was estimated using the detergent method of Mason (1969) which assumes that the only undigested dietary nitrogen is that associated with plant cell walls. This assumption has been found to hold true in studies of ruminants (Van Soest, 1967) and equines (Fonnesbeck, 1969) and the chemical technique yields similar estimates to the more traditional regression approaches. This was also the case in the present study but this may have been fortuitous since the confidence intervals of the regressions at zero nitrogen intake were wide. However, as Cork (1981) pointed out, the regression approaches assume that endogenous nitrogen excretion is constant and determined by a single dietary factor such as dry matter intake or faecal dry matter output. Since this is not always true (Mason and Frederiksen, 1979) there is no reason to suspect that the chemical partitioning is any less precise than the regression techniques.

The chemical technique is however, prone to inaccuracies from the contamination of faeces with hair ingested while grooming or from the presence of dietary protein-tannin complexes in the faeces. It was possible that some dietary nitrogen appeared in the faeces due to the action of tannins (Chapter 6), or as dietary nitrogen which is absorbed, recycled and not eventually reabsorbed. Therefore, this work follows Mason (1969) and Cork (1981) and terms the fraction not associated with cell walls as Non-Dietary Faecal nitrogen (NDFN).

Partitioning the faecal nitrogen losses of the Brushtail Possums further (Table 5.7) showed that NDFN losses on the eucalypt diet used here were much higher than on the semi-purified diets used by Wellard and Hume

TABLE 5.8: *Non-dietary faecal nitrogen excretion in several eutherian and marsupial herbivores (values gN.kg^{-1} dry matter intake)*

Species	Diet	NDFN	Reference
EUTHERIAN - Foregut fermenters			
Sheep	Hay (48-65% NDF)	4.5- 6.0	Colburn *et al.* (1968)
White-tailed and Mule Deer	Grass hay and pellets (42-64% NDF)	7.3- 8.3	Robbins *et al.* (1975)
White-tailed and Mule Deer	Browse (48-54% NDF)	9.0-10.1	Robbins *et al.* (1975)
Duiker, Eland, Hartebeest	Bran/concentrates (13% crude fibre)	3.5- 4.2	Arman *et al.* (1975)
- Hindgut fermenters			
Rabbit	Hay and concentrates (high fibre)	8.0	Slade and Robinson (1970)
Black-tailed Jackrabbit	Lucerne (high fibre)	4.6[1]	Nagy *et al.* (1976)
Snowshoe Hare	Browse and concentrates	6.1	Holter *et al.* (1974)
Rock Hyrax	Grain, fruit, beetpulp (low fibre)	3.7	Hume *et al.* (1980)
MARSUPIAL - Foregut fermenters			
Eastern Grey Kangaroo	Chopped oat hay and concentrates	3.7	Foley *et al.* (1980)
Euro }	Alkali extracted chopped oat hay and concentrates	2.7	Brown and Main (1967)
Tammar Wallaby	Chopped oat hay and sucrose	5.5	Hume (1977a)
- Hindgut fermenters			
Koala	Foliage (32-37% NDF)	5.9	Cork (1981)
Ringtail Possum	Foliage (51% NDF)	2.4	Chilcott and Hume (1984b)
Brushtail Possum	Semi-purified (17% NDF)	1.8	Wellard and Hume (1981a)
Brushtail Possum	Semi-purified (41% NDF)	3.4	Wellard and Hume (1981a)
Brushtail Possum	Foliage (27-30% NDF)	6.9	Present study
Greater Glider	Foliage (30-40% NDF)	5.0	Present study

[1]Calculated from data given.

(1981a) at similar levels of dietary NDF. However, the fibre source used in Wellard and Hume's (1981a) study was highly digestible. Several studies (Meyer, 1956, Whiting and Bezeau, 1957a, Wellard and Hume, 1981a) have shown that NDFN excretion is higher on higher fibre diets, but the source of fibre too has an important bearing (Whiting and Bezeau, 1957b). This effect may also explain the low MNRs of several of the species listed in Table 5.6. For example, the diets fed to the Euro (Brown and Main, 1968) were alkali extracted to reduce the content of lignin.

Since most of the NDFN consists of bacterial residues (Mason, 1969, 1971, Virtanen, 1966), the site of fermentation and the content of fermentable substrate in a diet will have an effect on NDFN excretion. Those animals that salvage bacterial residues from hindgut fermentation could be expected to excrete a smaller amount of NDFN. Mechanisms for retrieving some of this bacterial protein include caecotrophy; this probably explains the low NDFN excretion of the Ringtail Possum (see Table 5.8) compared with other herbivores. On the other hand, Slade and Robinson (1970) suggested that caecotrophy in rabbits and guinea pigs was responsible for the relatively high NDFN loss although no mechanisms were proposed to explain how this might occur.

An alternative method of retaining some of this microbial protein in the hindgut is by means of a "separation mechanism". Fine particles (Björnhag, 1972, Chapter 3) or solely bacteria (Sperber *et al.* 1983) can be preferentially retained in the caecum by these mechanisms. It is possible that the lack of an effective separation mechanism in the hindgut of the Brushtail Possum (Chapter 3) is responsible for the higher NDFN excretion compared to the three other folivorous marsupials in Table 5.8. The high MNR of the Brushtail Possum fed a diet of *Eucalyptus melliodora* foliage seems due mostly to its higher NDFN excretion. This in turn may be due to the lower digestibility of the eucalypt fibre compared to the semi-purified fibre source used by Wellard and Hume (1981a). In contrast, the high MNR of the Greater Glider is clearly due to its high urinary nitrogen excretion and the remainder of this discussion will be concerned with the reasons for this loss.

TABLE 5.9: *Parameters of urea kinetics in several marsupial and eutherian mammals fed low protein diets*

Species	Dietary crude protein (%)	Urea pool ($mgN.kg^{-0.75}.d^{-1}$)	Urea entry rate ($mgN.kg^{-0.75}.d^{-1}$)	Urea degradation rate		Reference
				$mgN.kg^{-0.75}.d^{-1}$	% entry rate	
EUTHERIAN - Foregut Fermenters						
Sheep	5	20	134	84	62	Cocimano and Leng (1967)
	9	135	363	158	4	Cocimano and Leng (1967)
Deer	5	164	1237	1099	90	Robbins *et al.* (1974)
	12	152	1001	619	62	Robbins *et al.* (1974)
Camel	3	914	1718	1610	94	Mousa *et al.* (1982)
	6	392	647	550	85	Emmanuel *et al.* (1976)
	10	415	777	572	74	Emmanuel *et al.* (1976)
EUTHERIAN - Hindgut Fermenters						
Horse	6	417	1024	665	65	Prior *et al.* (1974)
	9	473	1418	792	54	Prior *et al.* (1974)
Rock Hyrax	8	444	909	528	58	Hume *et al.* (1980)
Pig	8	91	251	62	25	Thacker *et al.* (1982)
MARSUPIAL - Foregut Fermenters						
Tammar Wallaby	10	125	505	271	55	Chilcott *et al.* (1984)
Red-necked Pademelon	10	156	509	322	64	Chilcott *et al.* (1984)
MARSUPIAL - Hindgut Fermenters						
Koala	8	134	352	270	78	Cork (1981)
Ringtail Possum	7	14	41	40	96	Chilcott and Hume (1984b)
Greater Glider	11	18	57	54	94	Present study
Brushtail Possum	8	82	153	91	59	Present study

Recent work on nitrogen metabolism in macropods (Chilcott *et al.* 1984) has shown that on medium protein diets, when water was freely available, Red-necked Pademelons recycled a similar proportion of their endogenously synthesised urea as did Tammar Wallabies. However, when water was restricted, the Pademelon's recycling efficiency dropped in contrast to the elevated recycling rates shown by the Tammars. In these experiments, creatinine excretion tended to be higher in the Pademelons than in other macropod marsupials (Hume, 1982) which tends to support Hume's (1977b) suggestion that there may be differences in the relationship between BMR and endogenous urinary nitrogen (EUN) among marsupials. An investigation of urea recycling in the Brushtail Possum and Greater Glider was clearly necessary to explain their different patterns of urinary nitrogen loss.

The level of urea metabolism in both the Greater Glider and the Brushtail Possums was low compared to most other herbivores fed low protein diets (Table 5.9). The principal exception to this was the Ringtail Possum (Chilcott, 1982) although sheep fed a low protein diet (Cocimano and Leng, 1969) had similar rates of synthesis and degradation of urea to those reported here for the Brushtail Possum.

Although the proportion of the urea synthesised that was recycled was higher in the Greater Gliders (90%) than in the Brushtail Possums (59%), this was offset by markedly lower rates of synthesis of urea in the Greater Gliders. From this perspective the total amount of urea-N recycled by the Brushtail Possums was 1.5 times that recycled by the Greater Gliders. The excretion rates of urea in the Greater Gliders were so low as to approach the limits of the analytical and statistical techniques used to derive the kinetic parameters. This in turn was due to the very low urinary content of urea-N (compared with total nitrogen) which was excreted.

There is a considerable body of evidence to suggest that the level of urea metabolism in ruminants is dependent on the extent of microbial activity. For example, Engelhardt *et al.* (1978) demonstrated low rates of urea recycling in starved goats. Varady *et al.* (1969) observed that the urea recycling rate varied with time after feeding in sheep, and Kennedy and

Milligan (1980) concluded that the rate of transfer of urea to the rumen was related to the fermentable energy content of the diet. Hence the relatively slow rate of urea synthesis in the Greater Glider and Brushtail Possum may be due to the relatively slow rate of fermentation in the hindgut (Chapter 9).

The nutritional significance of recycled urea may depend on the site at which it enters the gastro-intestinal tract. In ruminants, urea is degraded in both the caecum and the rumen with the majority of hindgut urea-N arising in the distal small intestine (Kennedy and Milligan, 1980). The transfer of urea to the fermentation regions is generally seen to benefit the animal by providing nitrogen for maintenance of the microbial population and hence, maintenance of fermentation rates (e.g. Chilcott and Hume 1984b). This interpretation is consistent with the evidence (above) suggesting a relationship between the level of urea metabolism and the extent of microbial activity. However, the quantitative importance of the transfer of urea to the rumen is in some cases only minor. For example, in some studies of ruminants fed low protein diets (Norton *et al.*, 1978, 1979, MacRae, 1979) the vast majority of the urea was degraded in the hindgut. Some of this nitrogen may be used for microbial protein synthesis while the remainder could be absorbed as ammonia and used to supply the liver with a source of nitrogen for anabolic purposes. In hindgut fermenters, the balance of evidence (Knutson *et al.*, 1977) suggests that while the distal small intestine is the major site of entry to the gastrointestinal tract, most of this urea is degraded in the caecum and/or proximal colon.

Cork (1981) concluded that nitrogen from degraded urea had little or no influence on microbial activity in the hindgut of Koalas since the supply of nitrogen from non-urea sources was probably adequate to support the measured rate of microbial activity. A similar calculation can be made here. If it is assumed that the stoichiometry of fermentation in the hindgut of the Greater Glider and Brushtail Possum is similar to that occurring in the rumen (ARC, 1980), then it can be calculated that 2.9 g nitrogen are incorporated into microbial protein per mole of short-chain fatty acids (SCFA) produced. Therefore in the Greater Glider, some 6.9 mmol of N would be fixed during the production of 33 mmol $SCFA \cdot kgW^{-0.75} \cdot d^{-1}$

(Chapter 9). However, NDFN excretion amounted to 15.7. mmol $N \cdot kgW^{-0.75} \cdot d^{-1}$ of which at least 14.8 mmol must have come from sources other than the degradation of urea in the hindgut. Similarly in the Brushtail Possum, about 8.3 mmol N would be fixed by microbial action but NDFN from non-urea sources was at least 18.6 mmol $N \cdot kgW^{-0.75} \cdot d^{-1}$. The extent to which this other nitrogen could have provided for microbial requirements would depend on the rates and extent of protease activity in the hindgut. However, it seems unlikely that microbial activity in the hindgut of either species was limited by nitrogen availability, even allowing for the possibility that *in vitro* SCFA production underestimated that *in vivo* (Chapter 9).

The proportion of degraded urea that is incorporated into microbial protein in the hindgut depends on a number of factors including the level of nitrogen intake and the water status of the animal (Hume *et al.* 1980). While no studies have been made in hindgut fermenters, there seems little reason to suspect that efficiency of capture of N would be markedly lower than in foregut fermenters. Values generally range from 40-60% (Hume *et al.* 1980) and in Tammar Wallabies at similar dietary N levels, between 60 and 70% of degraded urea was incorporated into microbial protein (Kennedy and Hume, 1978).

The low level of urea metabolism in the Greater Glider and Brushtail Possum (as well as in the Koala and Ringtail Possum), together with the lack of relationships between nitrogen intake and the rate of urea synthesis, suggests that in these species, other dietary factors were more important in determining the rate of urea synthesis in the body. Similarly, the very low quantities of urea excreted by the Greater Gliders and Ringtail Possums suggest that urea loss is not important in explaining their high rates of urinary nitrogen loss.

Partitioning of the urinary nitrogen of the Greater Glider showed that NH_4-N was the dominant fraction, comprising some 70-80% of total urinary nitrogen. Although it might be suspected that this situation arose from the hydrolysis of urea by bacterial ureases, the fact that the urea U/P ratio was greater than unity confirms that the observation is not due to urea breakdown. While there are many cases in which urea-N comprises

only a minor proportion of total urinary-N (e.g. Camel, Read, 1925; East African cattle, Elliot and Topps, 1963) the important point to note here is the magnitude of the urinary NH_4-N excretion.

Urinary NH_4-N is generally associated with the maintenance of body acid-base balance. Recently, conventional views of mammalian acid-base regulation have been challenged. Atkinson and Camien (1982) have suggested that disposal of metabolic base is a major problem for air-breathing animals. They further suggested that synthesis of urea is the mechanism by which this metabolic base is transported in and excreted from the body. At present there is little experimental evidence available to test the limits of this theory (Bean and Atkinson, 1984). Nonetheless, in view of the small effect of urea recycling on the nitrogen metabolism of the Greater Glider and Brushtail Possum, the following discussion will stress the points of divergence between this argument and the traditional viewpoint.

It was suggested (Chapter 1) that excretion of conjugated products in the urine was the principal mechanism for the detoxification of eucalypt terpenes and the simpler phenolics. The effect of conjugation of a xenobiotic which is perhaps only weakly acidic or even neutral, is to turn it into a relatively strong organic acid. The dissociation constants of a range of glucuronides, hippuric acids and phenaceturic acids are within the range 3.1-3.7 (Robinson *et al.*, 1953) and so all will be entirely ionized at physiological pH - certainly at the pH found in the urine of the Greater Glider. For example, the pKa of the monoterpene alcohol iso-menthol is about 18 while the pKa of iso-menthyl glucuronide is 3.7 (Robinson *et al.* 1953). In spite of Atkinson and Camien's (1982) arguments, animals that are producing large amounts of conjugated metabolites may well have an acid disposal problem.

The conventional view (e.g. Pitts, 1973) of the origin of urinary NH_4^+ is that it arises from the hydrolysis of glutamine (and to a lesser extent alanine) in the kidney to give glutamic acid and NH_3. The unionized ammonia passes into the lumen of the tubules where it combines with metabolic H^+ ions and the resulting NH_4^+ is excreted in the urine. Atkinson and Camien (1982) on the other hand show that catabolism of amino

acids (principally in the liver) leads to NH_4^+, not NH_3. These NH_4^+ ions combine with glutamate to form glutamine which is transported to the kidney and hydrolysed to glutamate and NH_4^+. Hence, NH_4^+ cannot facilitate the excretion of H^+ ions since NH_4^+ in the liver becomes NH_4^+ in the urine.

Atkinson and Camien (1982) propose that the urea cycle disposes of HCO_3^- as well as NH_4^+. They suggest that protons are "pumped" from NH_4^+ to HCO_3^- against an energy gradient. Therefore the excretion of acidic conjugated compounds in effect "titrates" some of the HCO_3^- that must be disposed of and urea production will fall accordingly. Residual NH_4^+ can then be excreted by actively secreting protons into the urine with the consequence that the concentration of NH_4^+ in urine exceeds that in blood.

The hypothesis proposed here to explain the high urinary nitrogen loss of the Greater Gliders and hence the high MNR is that NH_4^+ is excreted in the urine to regulate acid-base balance in connection with the excretion of detoxified allelochemicals. This hypothesis can be easily tested. If Greater Gliders were feeding on leaf that led to a lower excretion of glucuronides or some other detoxification product, then NH_4^+ would be expected to be lower and urea excretion higher. The short experiment with *Angophora floribunda* as the diet showed that the ratio of urinary urea:ammonia was a dietary effect and could be reversed. Since the *Angophora* leaf contained only traces of terpene and minor amounts of phenolics (W.J. Foley, unpublished), it may be inferred that this led to a lower excretion of conjugated compounds. Alternatively, *A. floribunda* may contain more carboxylic acids than *E. radiata* which, in Atkinson and Camiens (1982) view, would result in an increased urea excretion. However, these hypotheses can only be evaluated by experimental manipulation of the diets of the animals to provide them with known amounts of leaf allelochemicals or carboxylic acids. This, combined with measurements of urinary constituents and blood buffers, should give sound data on the extent of perturbation of body acid-base balance arising from the excretion of leaf terpenes and phenolics.

5.5 Summary

The high maintenance nitrogen requirement of the Greater Glider compared with other arboreal marsupials was due to the high urinary excretion of NH_4-N. This may have resulted from the need to balance the excretion of acidic conjugated xenobiotics. In contrast, excretion of NDFN was responsible for the higher MNR of the Brushtail Possum compared with previous estimates based on semi-purified diets. This high NDFN excretion was partly attributable to the lack of an effective digesta separation mechanism in the hindgut. Although both species recycled large proportions of urea to the gut, it was concluded that microbial activity was unlikely to have been limited by nitrogen availability.