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Frontispiece: Female koala with back-young
ENERGETICS AND NUTRITION DURING LACTATION IN THE KOALA, 
*PHASCOLARCTOS CINEREUS*:

HOW DOES AN ARBOREAL FOLIVORE MEET ITS ENERGY REQUIREMENTS FOR REPRODUCTION?

Andrew Karl Krockenberger BSc. (Hons I)

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

School of Biological Sciences

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August, 1993
Preface

The studies presented in this thesis were completed by the author while a postgraduate student in the School of Biological Sciences at the University of Sydney, Sydney, Australia. Assistance given by other persons is indicated in the text or in the list of acknowledgements. All references cited are included in a bibliography. The work is otherwise original.

I certify that the substance of this thesis has not already been submitted for any degree and is not being currently submitted for any other degree. I certify that any help received in preparing this thesis and all sources used have been acknowledged in the thesis.
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ABSTRACT

The energetics and nutrition of free-ranging female koalas was studied during lactation, at a site on the New England Tablelands of N.S.W.

The composition of koala milk was within the ranges found in other marsupials. However, the pattern of changes in composition differed in some aspects. Lipid was high throughout lactation and was the major source of energy even early in lactation.

Peak milk energy production in the koala, 99-122 kJ.kg⁻⁰·⁷⁵·d⁻¹, was the lowest measured for any mammal, but because of the long duration of lactation, the total requirement of the mother for metabolizable energy to support reproduction, 19-29 MJ.kg⁻¹, was within the range reported for other mammals. Thus, koalas meet the daily requirements of reproduction by spreading the load of reproduction over a long period, thereby minimizing daily requirements.

There were considerable differences in the output of milk energy by female koalas between the two years of the study. Total milk production was greater in 1990 than 1991. Consequently, both total and peak allocations of energy to reproduction by female koalas in 1991 were only 65 % of those in 1990.

Koalas met at least 90 % of the demands of their prolonged lactation by increasing food energy intake. They utilized very little, or no body energy stores to meet lactational requirements. Food intake of lactating females at the time of peak lactation was 27 % greater than that of non-lactating females during the same period. This increase in food intake at peak lactation was not associated with decreased retention time of digesta within the gut or with measurably increased dietary selectivity. The capacity of the gut for fluid digesta was 33 % greater in lactating females than non-lactating females; this allowed greater intake of foliage without a decrease in mean retention time of fluid and fine particulate digesta, and thus avoided the depression in digestibility and elevated loss of nitrogen in faeces usually associated with rapid passage of food.

Resting metabolic rates (RMR) of lactating females were greater than those of non-lactating females, but only 42 % of the expected level for synthesis of milk. Field metabolic rates (FMR) of both lactating and non-lactating females varied seasonally, with a nearly twofold increase from summer to winter. However, at the time of peak lactation, the FMR of lactating females was no greater than that of non-lactating females, despite the increased energy expenditure associated with synthesis and export of milk and with acquisition and processing of food (specific dynamic action).
export of milk and with acquisition and processing of food (specific dynamic action). These extra energy expenditures of lactating females could be expected to increase their energy budget by 270 and 420 kJ.d⁻¹ (in 1991 and 1990 respectively) which may be expected to have raised their FMR above the 95% confidence limits of that of the non-lactating females. That this did not occur clearly shows that lactating females compensated for part of the energy requirements of reproduction by reducing other aspects of their total energy expenditure.

Although lactating females had smaller home ranges than did non-lactating females, their low normal level of activity means that the reduction in home range size could not have compensated for any substantial proportion of reproductive energy expenditure. It is more likely that the reduction in home range served to reduce exposure of the potentially vulnerable females with back-young to terrestrial predators.

Some of the compensation for reproductive energy demands may have occurred in thermoregulatory expenditure. By carrying a juvenile on the back or belly, lactating females effectively reduced their surface area by about 10%, saving up to 108 kJ.d⁻¹ in thermoregulatory expenditure. However, some of the compensation was due to a reduction of some component of the RMR, as it rose by only 42% of that expected. Thus there were multiple sources of compensation for reproductive energy expenditure.

Koalas met the energy demands for reproduction in two ways. First, by spreading lactation over a long period, they minimized daily energetic demands; compensation for reproductive energy expenditure by reduction of other components of resting and field metabolic rates reduced daily reproductive demands even further. Second, by increasing gut capacity they were able to increase food energy intake to meet energy demands.
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CHAPTER ONE

THE DEMANDS OF REPRODUCTION IN MAMMALS

1.1 INTRODUCTION

"It would be instructive to know not only by what physiological mechanism a just apportionment is made between the nutriment devoted to the gonads and that devoted to the rest of the parental organism, but also what circumstances in the life-history and environment would render profitable the diversion of a greater or lesser share of the available resources towards reproduction."

Fisher (1930)

The demands of reproduction are central to our understanding of many processes in ecology and evolution. The division of the finite resources available to an organism into reproduction or other functions has attracted interest since Fisher (1930) drew attention to its ramifications for life-history and ecology. There have been two major approaches to the study of resource-partitioning during reproduction. Evolutionary biologists have concentrated on the "cost" of individual reproductive events to future reproduction and physiologists on the energetic "effort" devoted to reproduction. While both these approaches are essential to our understanding of the effects of reproduction on the ecology of organisms, few studies have integrated the two concepts.

The demands of reproduction and their links with the ecology of an arboreal folivore, the koala (Phascolarctos cinereus), are the theme of this study. In these first two chapters I aim to outline the concepts of reproductive energetics, strategies among mammals, the unique position of the koala and other arboreal folivores, and the expected effects of reproductive demands on the ecology of koalas.

1.2 COST AND EFFORT: EVOLUTION AND ECOLOGY

Many studies of reproductive energetics are couched in terms of evolutionary or life-history theory. Because of this, I briefly outline the two key concepts of reproductive "costs" and "effort".

The "cost" of reproduction is a concept introduced by Williams (1966) to explain the apparent restraint shown in reproduction by many organisms, e.g. late maturation, low
fecundity and long life-span among sea birds (Wynne-Edwards 1955). Williams(1966) defined it;

"The cost of a reproductive function is any quantitative decrement in either the effectiveness of another function, or the probability of surviving to perform that function, or both." The second function referred to is usually taken to be that of future reproduction (Bell, 1980). Thus reproductive "cost" is the negative causal effect that expenditure on current reproduction has on residual reproductive value, resulting in a trade-off between current and future reproduction (Pianka, 1976; Calow, 1979; Bell, 1980; Steams, 1989).

Reproductive "effort" is an energetic measure of the resources devoted to reproduction compared to the resources devoted to somatic purposes (Fisher, 1930). Most investigations of reproductive "costs" since the 1930's have used energy expenditure, "effort", as the currency in the trade-off between current reproduction and residual reproductive value (Williams, 1966; Pianka, 1976; Calow, 1979; Tuomi et al., 1983), due to the difficulty of measuring reproductive "cost" (Calow, 1979; Knapton, 1984). This has led to some confusion between the terms reproductive "costs" and "effort" (Bell, 1980). Many authors have used the terms "effort" and "cost" interchangeably, referring to energetic costs of reproduction (e.g. Thomson et al., 1970; Randolph et al., 1977; Millar, 1978; Havaera, 1979; Lochmiller et al., 1982; Harvey, 1986; Anderson and Fedak, 1987; Racey and Speakman, 1987; Carl and Robbins, 1988; Konig et al., 1988).

The link between "cost" and "effort" has rarely been demonstrated (Calow, 1979; Tuomi et al., 1983; Clutton-Brock, 1984; Reznick, 1985, 1992) especially in long lived organisms. Hence, energetic measures are of limited use in evolutionary or life-history theory (Bell, 1980; Clutton-Brock, 1984; Reznick, 1985, 1992; Shine and Schwarzkopf, 1992).

Despite the limited value of physiological measures of energetics to evolutionary or life-history theory, they are the most appropriate measure in explaining shorter-term phenomena, such as the relationship of reproductive requirements to the ecology of the organism. These include food and energy requirements (Gittleman and Thompson, 1988), and the allocation of energy to reproduction (Randolph et al., 1977; Millar, 1978; Racey and Speakman, 1987; Kenagy et al., 1990), as well as the relationship between the size of litter and size of young (e.g. Millar, 1975; Tuomi, 1980 ; Mattingly and McClure, 1982; Glazier, 1985). Energetic requirements also explain changing habitat use and behaviour during reproduction (Clutton-Brock et al.,
1982,) and the relationship of habitat quality to short-term reproductive success (Clutton-Brock et al., 1982; Loudon et al., 1984).

This study is an ecophysiological approach to describing and explaining the allocation of energy to reproduction in the koala, and relating this to habitat requirements and use during this time, as well as to the peculiar mode of life of the koala. As such I deal with the relation of this to life-history and evolution only peripherally, but instead concentrate on energetic measures of reproductive effort, their relation to arboreal folivory, and their ramifications for the ecology of the koala.

1.3 DEMANDS OF DIFFERENT STAGES OF REPRODUCTION

1.3.1 Mating

Most studies of energy use during mating have concentrated on behavioural measures of male mammals, with some indication that mating can be energetically expensive (Gittleman and Thompson, 1988). The most extreme example in mammals is that of several Antechinus species where males are semelparous, and die after the rut, but at least a proportion of the females are iteroparous (Braithewaite and Lee, 1977; Lee and Cockburn, 1985). The intensive rut is accompanied by large increases in corticosteroid hormones (Bradley et al., 1980), followed by death of all males (Lee and Cockburn, 1985). The hormones presumably both enhance the males competitive ability during the rut (by increasing aggression and diverting resources from somatic metabolism to reproduction) and subsequently kill them by somatic debilitation and suppression of the immune system (Lee and Cockburn, 1985). The males reduce or suspend other activities during the rut, feeding little (Lee and Cockburn, 1985), and devote themselves to mating and aggressive encounters. Despite the fact that energy use does not increase (Lee and Cockburn, 1985), reproductive "effort" (sensu Tuomi et al., 1983) is great due to the diversion of energy from somatic stores. Post-mating population decline in the vole, Microtis townsendii, seems to have a similar mechanism (Krebs and Boonstra, 1978).

There is other evidence that mating may be energetically demanding for males of a number of species. In grey seals (Halichoerus grypus), the largest males enjoy a success in achieving copulations ten times that of small males but lose weight faster and stay on the beach longer, indicating a greater use of stored energy (Anderson and Fedak, 1987). During the rut, male red deer increase non-feeding activities at the expense of feeding (Clutton-Brock et al., 1982), and weight loss can be considerable. Males also spend time and energy advertising and competing and keeping track of
females. Male koalas bellow for several months during mating (Mitchell, 1990a), have higher levels of activity, larger home ranges, and overlap spatially with a larger number of females than do female koalas (Mitchell, 1990a,b; Mitchell and Martin, 1990). Presumably these activities are both risky in terms of accident and predation, and energetically demanding. These sorts of patterns are common in male mammals and have been considered to be the consequence of the mammalian mode of reproduction which necessitates direct energy transfer from mother to young but no actual transfer from the male (Daly, 1979).

Other aspects of energetics of mating are more difficult to quantify and their effects may extend over greater periods than mating. Male golden-mantled ground squirrels (*Spermophilus saturatus*) arouse from hibernation a week earlier than females, to prepare for mating by marking and defending an area in which they later mate (Kenagy, 1987). In species with marked sexual dimorphism the male is usually larger (Ralls, 1976), and so has a greater absolute energy requirement throughout the year (Nagy, 1987). There is also an energetic demand involved with the production of sperm (Dewsbury, 1982). There may also be an energetic demand involved in establishing and maintaining dominance hierarchies. In European rabbits high ranking males, which also have high reproductive success, have higher resting metabolic rates than subordinates (Bell, 1986), though in shrews and deer mice the pattern is reversed (Farr and Andrews, 1978; Fuchs and Kleinknecht, 1986), making extrapolation from these examples to other species difficult. A different pattern, but one still difficult to quantify, occurs in monogamous species where males either forgo the opportunity to seek other mates, defend the female, or participate in rearing the young (Daly, 1979).

Only two studies have directly measured the energy expended by male mammals during mating. Those two studies, of *Antechinus* (Lee and Cockburn, 1985) and golden-mantled ground squirrels (Kenagy, 1987), show little increase above normal in overall energy expenditure during mating. However, this could be partly due to the difficulty of separating the relatively brief periods of mating from other components of the energy budget and partly because of diversion of resources from somatic processes (e.g. in *Antechinus*, discussed above).

It is difficult to partition the energy input of female mammals to mating from more long-term preparation for reproduction such as fat storage (Pond, 1984). Gittleman and Thompson (1988) suggested that correlations of stress and resource limitation with decreases in fecundity (Loudon, 1987b; Thomas, 1982; Ward, 1990) show that there is some energetic effort at this time, but less than in males. However, these
correlations could simply indicate a low risk breeding strategy, as reproduction could fail under later conditions of resource limitation.

1.3.2 Gestation and lactation

"we should perhaps consider the cow as an appendage to the udder rather than the reverse"


Gestation and lactation are the periods of reproduction that place the most obvious demands on female mammals. Maternal input to reproduction has been gauged in three ways; the duration, litter size and weight, and the energy input. The first two are primarily intended to describe aspects of the third, energetics, which is the major theme of this section.

1.3.2.1 Duration

Life-history parameters, as well as metabolism, are correlated with body mass (Kleiber, 1975; Millar, 1977; Blueweiss et al., 1978; Henneman, 1983; Peters, 1983; Schmidt-Nielsen, 1984; Martin and Maclamon, 1985; McNab, 1980, 1986, 1987, 1992; Elgar and Harvey, 1987; Nagy, 1987). Therefore, comparison of life-history or of metabolic parameters between animals of different size must consider what component of differences is allometric.

Among eutherian mammals the duration of gestation is related to maternal mass (Equation 1.1),

\[ t_g = 62.99 M_m^{0.238} \quad \text{Equation 1.1} \]

where \( t_g \) is the gestation period in days and \( M_m \) is the maternal mass in kg (Millar, 1981). Millar (1981) demonstrated a positive relationship between gestation length and individual and litter weights, but a negative correlation with litter size (Equation 1.2). He suggested that this was due to physical and physiological constraints associated with "crowding" in utero.

\[ \log_{10} t_g = 4.637 + 0.190 M_m - 0.163 N \quad \text{Equation 1.2} \]

where \( N \) is the litter size. Sacher and Staffeldt (1974) found gestation period to scale similarly with neonatal and maternal mass and brain weight. However, brain weight measures gave a better fit than body weight, so they concluded that brain growth was the limiting process in gestation (Sacher and Staffeldt, 1974).
In marsupials, gestation ranges from 12 to 46 days (Russell, 1982a). Only in large (macropodid) marsupials is the gestation period markedly shorter than in eutherians (Tyndale-Biscoe, 1973), though marsupial neonates are in many respects markedly more altricial than eutherian neonates, making the marsupial reproductive strategy far more dependent on lactation (Russell, 1982a; Lee and Cockburn, 1985). However, the gestation period does not seem to be closely related to maternal mass in marsupials (McNab, 1986), though Russell (1982a) questioned the utility of gestational duration as a comparative measure due to the varying periods of pre-attachment quiescence (or diapause) of the embryo in a variety of mammals. The significance of gestation period and other differences in reproductive strategy between eutherian and marsupial mammals have been disputed in the literature for a number of years (Section 1.5).

Millar's (1977) review of the duration of lactation in eutherians did not show a significant relationship of lactational duration with maternal mass. Lactation lasted for 12-70 days and variation in maternal mass explained only 4% of the variation in the time from conception to weaning (Millar, 1977). This may be partly due to problems in determining the time of weaning (Millar, 1977; Russell, 1982a) and the predominance of small mammals in the data set, but may also indicate that the duration of lactation is influenced by factors other than maternal mass. Certainly there is great variability in the duration of lactation within some groups of eutherians (a range of at least 300 days in similarly sized pinnipeds, Bonner, 1984). However, other analyses of the length of lactation in eutherians (as well as most other physiological processes) suggest that it is proportional to maternal mass to an exponent of 0.25-0.27 (i.e. $M_m^{0.25}$ to $0.27$) (Taylor and Murray, 1987). Taylor and Murray (1987) suggest that the duration of lactation among eutherians can be expressed as 50 metabolic days ($\{\text{days-3.5}\}.M_m^{-0.27}$).

The duration of lactation in marsupials is correlated with maternal mass,

$$t_l = 35.7 M_m^{0.22} \quad \text{Equation 1.3}$$

where $t_l$ is the duration of lactation in days, and $M_m$ is the maternal mass in g (Russell, 1982a). Because of the different reproductive strategies of eutherians and marsupials, the time from conception to weaning ($t_{cw}$) is a more appropriate measure of the duration of a female mammal's reproductive demands for comparison than gestation or lactation alone (Lee and Cockburn, 1985).
Lee and Cockburn (1985) compared the time from conception to weaning between eutherian and marsupial carnivores and omnivores (except bandicoots), deriving the following allometric relationships:

\[ t_{cwE} = 99.7 M_m^{0.17} \]  \hspace{1cm} \text{Equation 1.4}

\[ t_{cwM} = 151.4 M_m^{0.093} \]  \hspace{1cm} \text{Equation 1.5}

where \( t_{cwE} \) is the time from conception to weaning for eutherians in days, \( t_{cwM} \) is the same for marsupials and \( M_m \) is the maternal mass in kg. Combining the two equations they showed that the \( t_{cw} \) is greater for marsupials than eutherians (within carnivores and omnivores), and that the difference is greatest for small maternal masses (Equation 1.6, Lee and Cockburn, 1985).

\[ t_{cwM} = 1.52 M_m^{-0.076} t_{cwE} \]  \hspace{1cm} \text{Equation 1.6}

S. Thompson (1987) extended the comparison between \( t_{cw} \) in marsupials and eutherians to include herbivorous marsupials, and also found that \( t_{cw} \) was greater in marsupials than eutherians, except in the size range 1-3.5 kg. This size range includes the marsupial peramelids and didelphids, noted for their rapid maturation (Lee and Cockburn, 1985). Thus the duration of parental care is longer in marsupials than eutherians at large and small body masses (S. Thompson, 1987). The flexibility in \( t_{cw} \), indicated by short times in the omnivorous peramelids and didelphids, suggests that like \( t_l \) in eutherians (see above) \( t_{cw} \) in marsupials may be affected by ecological factors as well as by mass.

The duration of lactation can be affected by food availability. A low plane of maternal nutrition is associated with early weaning in mice (Konig and Markl, 1987), but extended lactation in some primates (Lee et al., 1991), seals (Trillmich, 1986) and elephants (Lee and Moss, 1986). Extending lactation, with little change to the total output, would reduce the daily energy requirements for lactation (Glazier, 1985,1990a,b; Cork and Dove, 1989; Lee et al., 1991).

### 1.3.2.2 Production

**a) Gestation**

Foetal growth in eutherian mammals follows near to a sigmoid curve (Payne and Wheeler, 1967; Martin, 1984; Martin and McLaron, 1985) or the similar Gompertz Equation (ARC, 1980). That is, the foetus grows slowly at first, then rapidly, but until late in the gestation is relatively small with little energy requirement compared
with that of the mother. Thus the highest energetic demands of gestation do not occur until late in the gestation (Oftedal, 1985; Weiner, 1987a; Speakman and Racey, 1987; Prentice and Whitehead, 1987).

The energy required for gestation is a function of the mass of foetus produced, the placental and uterine production and the efficiency of production (13.3 % for domestic ruminants, ARC, 1980).

The relationship of production in gestation (i.e. neonatal mass and energy content) to the body mass of the mother has been described a number of times (Robbins and Robbins, 1979; Millar, 1981; Martin, 1984; Martin and McLarnon, 1985; Oftedal, 1985). For small mammals especially, the occurrence of different litter sizes with nonlinear effects on the litter mass (Millar, 1981) complicates the consideration of patterns of production in gestation. Production during gestation in eutherian mammals is related to maternal mass and litter size (Millar, 1981), although carnivores and edentates have relatively low production.

\[
\log_e M_b = 0.826 \log_e M_m - 0.206 N - 2.470 \quad \text{Equation 1.7}
\]

\[
\log_e M_{lb} = 0.797 \log_e M_m + 0.101 N - 2.501 \quad \text{Equation 1.8}
\]

Where \( M_b \) is the individual neonatal mass in kg, \( M_m \) is the non-reproductive maternal mass in kg, \( M_{lb} \) is the neonatal litter mass in kg, and \( N \) is the litter size.

Precocial and altricial eutherian mammals differ in allocation of energy to gestation. Altricial mammals usually produce large litters of small offspring (Martin and McLarnon, 1985). With large litters the gestation period and neonatal mass are reduced (Millar, 1981), but litter mass increases so that altricial mammals have higher total and daily allocations of energy to gestation than do precocial mammals (Millar, 1981; Martin, 1984; Martin and McLarnon, 1985).

The relative production of young in gestation (\( M_l/M_m \)) decreases as maternal mass increases (Robbins and Robbins, 1979; Oftedal, 1985). In ungulates and subungulates the range is from 16 % in the pronghorn antelope to about 3% in the hippopotamus (Robbins and Robbins, 1979). Presumably this relates to a higher energetic allocation to gestation in small than in large mammals. The small bats have relatively high birth weights and presumably relatively high energetic requirements for gestation (Kurta and Kunz, 1987) while the large bears have low birth weights and requirements for gestation (Millar, 1981; Ramsay and Dunbrack, 1986).
In marsupials, production in gestation is also related to maternal mass (Russell, 1982a).

$$M_b = 0.0003 M_m^{0.56} \quad \text{Equation 1.9}$$

$$M_{lb} = 0.023 M_m^{0.35} \quad \text{Equation 1.10}$$

Where $M_b$ is the individual neonatal mass in kg, $M_m$ is the non-reproductive maternal mass in kg, $M_{lb}$ is the neonatal litter mass in kg, and $N$ is the litter size.


$$M_{IE} = 428.7 M_m^{0.49} M_{IM} \quad \text{Equation 1.11}$$

Where $M_{IE}$ and $M_{IM}$ are the neonatal masses of eutherian and marsupial litters respectively.

Production by marsupials in gestation is considerably less than by eutherians (Equation 1.11). No marsupial litter weighs more than 1% of maternal mass at birth (Russell, 1982a), and among eutherians only bears have such a low birth mass (0.33% in Ursus americanus, Millar, 1981). Thus, based on litter mass, it is likely that marsupials allocate much less energy to gestation than do eutherians, and that the quantity is likely to be far less significant to their ecology. However, production of neonatal mass in gestation does not account for the increases in maternal metabolism required to synthesize and maintain foetal and placental tissue - the heat increment of gestation (Brody, 1945). Increases in maternal metabolism during gestation are generally not large, possibly due to forms of energetic compensation in some species, but may be significant in some species with low basal metabolic rates (BMR) (Nicoll and Thompson, 1987, Section 1.4.2).

Gestation represents a variable proportion of a successful reproduction and lactation is generally more costly (Clutton-Brock et al., 1989), as well as more energetically demanding (Section 1.4.1), so investment in gestation is only a small part of maternal investment.
b) Lactation

Production during lactation in eutherians has been assessed by the weight of young at weaning minus the birth weight (Millar, 1977).

\[ M_{w-b} = 0.83 \, M_m^{0.7} \] \hspace{1cm} \text{Equation 1.12}

where \( M_{w-b} \) is the mass of one young at weaning minus neonatal mass in g and \( M_m \) is maternal mass in g.

In marsupials, the mass at birth is small enough to ignore in the production of young during lactation (Russell, 1982a).

\[ M_{w-1} = 0.47 \, M_m^{0.96} \] \hspace{1cm} \text{Equation 1.13}

where \( M_{w-1} \) is the mass of the litter at weaning in g.

Clearly, there are differences between marsupials and eutherians in the production of mass of young in lactation. However, these are largely due to the differences in proportion of total production made during gestation in marsupials and eutherians. Therefore, the most appropriate measure of production of young for comparison between the two groups is the total production in both gestation and lactation. Lee and Cockburn (1985) showed that the relative masses of offspring and mother were similar between marsupials and ecologically comparable eutherians. Millar (1977) proposed that the mass of the young at weaning in mammals represented the minimum mass for survival in the adult niche. If this is so then it is not surprising that marsupials and eutherians wean similar sized young.

However, the size of young at weaning does not accurately describe the energetic input of the mother to those young, especially in herbivores that have a long period of weaning, because during weaning the young ingest energy from sources other than milk. Size of young at weaning also does not account for the increases in maternal metabolism (e.g. Thompson and Nicoll, 1986; Nicoll and Thompson, 1987) required for the production of milk and maintenance of the mammary gland- the heat increment of lactation (Brody, 1945, Section 1.4.2). The yield of milk energy is a more direct description of the allocation of energy to lactation. Yield of milk energy must also be corrected for other costs of production of that milk, including metabolic efficiency, foraging and carrying the young, before a true measure of maternal energy input during the lactation can be obtained.
Milk production generally rises to a peak after parturition and then declines exponentially until the termination of weaning. In dairy cattle the pattern of milk production can be described by a curve of the form:

\[ M = a t^b e^{-ct} \]  

Equation 1.14

where \( M \) is milk production, \( a, b, c \) are constants and \( t \) is the time (Wood, 1977).

The magnitude of \( b \) sets the rate of the initial increase, that of \( c \) sets the rate of the exponential decrease, and \( a \) is the initial milk production. In the only marsupial for which the form of the lactation curve has been examined in detail, *Macropus eugenii*, an equation of the form given in Equation 1.12 fitted well to the milk production around and after the period of pouch emergence (Dove and Cork, 1989). However, due to the long initial period (Phase 2a, Tyndale-Biscoe and Janssens, 1988) of low milk production in *M. eugenii*, a more complex, composite equation was required to describe the entire lactation curve (Dove and Cork, 1989). Because of differences in the form of the lactation curve, comparison of milk production between species has generally been made on the basis of the magnitude of peak milk production (Table 1.1).

### Table 1.1 Peak milk energy yield of eutherian mammals.

<table>
<thead>
<tr>
<th>Type of mammal</th>
<th>( E_{pl} ) (kJ.d(^{-1}))(^{A} )</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>All mammals</td>
<td>( E_{PL} = 527 M_{m}^{0.73} )</td>
<td>Linzell (1972)</td>
</tr>
<tr>
<td>Non-dairy</td>
<td>( E_{PL} = 606 M_{m}^{0.69} )</td>
<td>Linzell (1972)</td>
</tr>
<tr>
<td>Non-dairy</td>
<td>( E_{PL} = 532 M_{m}^{0.69} )</td>
<td>Hanwell and Peaker (1977)</td>
</tr>
<tr>
<td>Non-dairy</td>
<td>( E_{PL} = 598 M_{m}^{0.65} )</td>
<td>Oftedal (1984)</td>
</tr>
<tr>
<td>Ungulates</td>
<td>( E_{PL} = 987 M_{m}^{0.52} )</td>
<td>Robbins and Robbins (1979)</td>
</tr>
</tbody>
</table>

\(^{A}E_{PL} \) is the milk energy output at peak lactation in kJ.d\(^{-1}\) and \( M_{m} \) is the maternal mass in kg.

Milk energy output at peak lactation in eutherians is proportional to maternal mass raised to an exponent close to 0.7 (Table 1.1) and not significantly different to 0.75 (Oftedal, 1984). Thus, milk energy yield at peak lactation is generally accepted to be proportional to \( M_{m}^{0.73} \) or 0.75 (Oftedal, 1984; Taylor and Murray, 1987; Cork and Dove, 1989).

Oftedal (1984, 1985) suggested that published values of milk energy output must be viewed with some caution, asserting that data in the literature was of uneven quality.
due to methodological differences in the measurement of milk yield (Oftedal, 1984). Also, milk composition varies considerably over the course of lactation (Oftedal, 1984), especially in marsupials (Green, 1984), as well as with time during a sampling (Oftedal, 1984). Failure to appropriately standardize methods and control for stage of lactation can lead to errors in the estimation of energy output at peak lactation. Thus, Oftedal (1984) rejected most published values of milk composition and yield (except those for 56 (composition) and 21 (yield) species). However, despite the reduction in the size of the dataset his relationship was similar to those previously derived and not statistically different from those of Linzell (1972) (Table 1.1).

Nevertheless, there is considerable deviation from these relationships, with peak yield ranging from 146 to 1029 kJ.M⁻⁰.⁷⁵.d⁻¹ (Oftedal, 1984). As with the relationship of neonatal mass to maternal mass, several grades exist within the yield of milk. Species with many young, such as carnivores (except for bears, Gittleman and Oftedal, 1987), produce high quantities of milk energy, and primates with single young produce much less (Oftedal, 1984).

Because the daily milk energy output in lactation is proportional to \( M_m^{0.75} \) (Oftedal, 1984) and the duration of lactation is proportional to \( M_m^{0.25} \) (Linstedt and Calder, 1981; Taylor and Murray, 1987), the total energy output of lactation (i.e. the area under the lactation curve) should be proportional to \( M_m^{-1} \) \( (M_m^{0.75}.M_m^{0.25}) \) (Taylor and Murray, 1987; Cork and Dove, 1989).

At present there are not enough data available to empirically determine the allometric scaling of total milk yield with \( M_m \), because total lactational output is difficult to measure due to the long tail of the exponential decay phase of the lactation curve (Equation 1.12), as well as changes in the composition and hence energy content of the milk. In an effort to avoid these problems, Oftedal (1985) derived a relationship of the total yield during "effective lactation" (excluding very small daily milk production in the terminal portion of the curve) to maternal mass in seven ungulate species, using an approximation to the average energetic content of the milk.

\[
E_{TL} = 25.06 M_m^{0.81}
\]  

Equation 1.15

where \( E_{TL} \) is the total milk energy of "effective lactation" in MJ.

However, the relationship derived by Oftedal (1985) (Equation 1.15) is based on a small data set with several assumptions, and so may not be representative of all mammals.
Milk energy output related to the mass of young better than maternal mass

\[ E_{PL} = 941 M_y^{0.83} N \]  \hspace{1cm} \text{Equation 1.16}

where \( M_y \) is the mass of an individual young and \( N \) is the litter size (Oftedal, 1984).

The relationship of milk energy output to mass of young (Equation 1.16) had less residual variation than that of output to maternal mass (Table 1.1), ranging from 640 to 1190 \( \text{kJ}.M_y^{-0.83}.d^{-1} \) (Oftedal, 1984). Oftedal (1984) termed \( M_y^{0.83} N \) the litter metabolic mass, suggesting that the demands of the young set the peak milk energy output.

In domestic ruminants, and possibly other eutherians, the demand for milk greatly influences total production. Both total and peak milk energy yield of dairy cows can increase 30 to 40% when milk is removed from the mammary gland three times daily rather than twice (Henderson et al., 1985). This is probably due to removal of an inhibitor of milk-secretion that is found in milk (Henderson et al., 1985). In marsupials however, milk production seems to be controlled by the mother. Results of intra- and interspecific swaps of pouch-young macropods showed that milk production did not increase with greater demands, leading to retarded growth of young transplanted to the pouch of a female formerly carrying a smaller young (Merchant and Sharman, 1966; Green et al., 1988).

Milk production has not been measured for enough marsupial species to be able to determine allometric relationships of yield with mass. However, both those measured had low peak milk energy output; 26% and 40% of that expected for eutherians (Table 1.1, Oftedal, 1984) in *Pseudocheirus peregrinus* (Munks, 1990) and *Macropus eugenii* (Cork and Dove, 1989) respectively.

Within and between species, energy input to individual young may be less for females with large litters than for females with small litters (Smith and McManus, 1975; Millar, 1975, 1978, 1979; Randolph et al., 1977; Innes and Millar, 1979; Lochmiller et al., 1982), e.g. ungulates with twins produce more milk than those with singletons, but not twice as much (Robbins and Robbins, 1979; Sadleir, 1982). However, several studies of rodents have found no relationship of litter size and offspring growth (presumably an index of milk production) or size at weaning (Millar, 1973; McClure and Randolph, 1980; Mattingley and McClure, 1982).
1.3.3 Post-lactation

Energy requirements of the post-lactational period are difficult to define precisely. They include parental care of the young and replacement of energy stores utilized during earlier stages of reproduction (Gittleman and Thompson, 1988), and possible competition for resources with the independent young (Lee and Cockburn, 1985; Cockburn and Johnson, 1988).

Pollock (1979) suggested that male subordinance to females at feeding sites in lemurs was a form of reproductive investment by the males, increasing the resources available for reproduction to the females and hence presumably increasing their own reproductive success. Lee and Cockburn (1985) considered competition of weaned but resident young with the parents to be part of the "costs" of reproduction, and suggested that this was one of the factors influencing the evolution of patterns of differential investment in male and female offspring and of dispersal patterns.

1.4 STRATEGIES FOR MEETING REPRODUCTIVE DEMANDS

There are several ways in which mammals meet the demands of reproduction. In most species more than one of these strategies operates concurrently, making it difficult to assess the precise input to reproduction from any one source (Millar, 1975; Randolph et al., 1977; Clutton-Brock et al., 1982; Sadleir, 1984; Speakman and Racey, 1987; Weiner, 1987a; Clutton-Brock et al., 1989). The major strategies are:

1) Increased assimilation- this is a combination of increased energy intake and/or increased efficiency of assimilation of that energy (Section 1.4.1).

2) Metabolic changes- such as increases in maternal metabolism for production of milk or metabolic compensation for the demands of reproduction (Section 1.4.2).

3) Changes in maternal energy stores- the storage and use of fat (Section 1.4.3).

1.4.1 Increased Assimilation (Strategy 1)

Most mammals meet the majority of the energy requirements of reproduction by increasing their intake of energy (Table 1.2), although other strategies may also contribute to varying degrees (Sections 1.4.2 and 1.4.3).
Because foetal growth follows a sigmoid form (Section 1.3.2.1), the energetic demands of gestation increase sharply toward term. Maternal food energy intake also reaches a peak just before parturition. This peak of intake represents the maximum energy flux that the mother requires from her environment during gestation. The average increase in intake over the entire gestation is only a small fraction of the peak (Oftedal, 1985; Prentice and Whitehead, 1987).

There are also a number of studies that have recorded little (in shrews, Genoud and Vogel, 1990) or no increase in food energy intake during gestation (in humans, Prentice and Whitehead, 1987; *Peromyscus maniculatus*, Stebbins, 1977), or even a decrease immediately before parturition (in sheep, Hadjipieris and Holmes, 1966; rabbits, Partridge *et al.*, 1986).

The energetic requirements for gestation in marsupials are largely unknown but are assumed to be small due to the brief gestation periods and tiny neonatal mass.

In summary, gestation places varying demands on different mammal species. The demand is greatest near to parturition and is usually met by an increase in food energy intake, although other factors such as energy storage and changes in maternal respiration can be important (Sections 1.4.2 and 1.4.3). Even when food intake is the primary source of energy for gestation the increase is relatively low, from about 20 to 30% (Table 1.2).

Lactation places greater demands on maternal metabolism, with peak lactation being the most crucial period (Millar, 1977; Gittleman and Thompson, 1988). Intake usually increases considerably more during lactation than gestation (Table 1.2). Part of the large difference in increases in intake among species is due to differences in the amount of maternal energy stores utilized during lactation (Sadleir, 1984). Species in which intake during lactation is not increased, or is zero, such as those pinnipeds which fast (Bonner, 1984; Ortiz *et al.*, 1984; Costa *et al.*, 1986) and bears that "den" during lactation (Ramsay and Dunbrack, 1986; Watts and Hansen, 1987), rely entirely on maternal stores for the energy requirements of lactation (Section 1.4.3), while others rely on stored energy to differing extents (Sadleir, 1984).
Table 1.2 Peak increase in energy intake during gestation and lactation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mass (g)</th>
<th>Increased intake (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gestation</td>
<td>Lactation</td>
</tr>
<tr>
<td><em>Peromyscus polionotus</em></td>
<td>13</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td><em>P. maniculatus</em></td>
<td>15</td>
<td>88</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>134</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>194</td>
<td>3</td>
</tr>
<tr>
<td><em>P. leucopus</em></td>
<td>22</td>
<td>29</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>56</td>
<td>1</td>
</tr>
<tr>
<td><em>P. eremicus</em></td>
<td>22</td>
<td>66</td>
<td>1</td>
</tr>
<tr>
<td><em>P. floridanus</em></td>
<td>42</td>
<td>35</td>
<td>1</td>
</tr>
<tr>
<td><em>Clethrionomys glareolus</em></td>
<td>20-25</td>
<td>24</td>
<td>5</td>
</tr>
<tr>
<td><em>Microtis arvalis</em></td>
<td>23-30</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td><em>M. pinetorum</em></td>
<td>29</td>
<td>48</td>
<td>7</td>
</tr>
<tr>
<td><em>Phodopus sungorus</em></td>
<td>30</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td><em>Mus musculus</em></td>
<td>30</td>
<td>31-80.9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
<td>10</td>
</tr>
<tr>
<td><em>Rattus norvegicus</em></td>
<td>100</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>110-200</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td><em>Sigmodon hispidus</em></td>
<td>126</td>
<td>66</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>110-200</td>
<td>111</td>
<td>16</td>
</tr>
<tr>
<td><em>Neotoma floridana</em></td>
<td>184</td>
<td>65</td>
<td>17</td>
</tr>
<tr>
<td><em>Spermophilus saturatus</em></td>
<td>232</td>
<td>90</td>
<td>18</td>
</tr>
<tr>
<td><em>Sicarius niger</em></td>
<td>875</td>
<td>149</td>
<td>19</td>
</tr>
<tr>
<td><em>Marmota flaviventris</em></td>
<td>2400</td>
<td>92</td>
<td>20</td>
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<tr>
<td><em>Myocastor coyopus</em></td>
<td>6360</td>
<td>63</td>
<td>21</td>
</tr>
<tr>
<td><em>Oryctolagus cuniculus</em></td>
<td>4223</td>
<td>100</td>
<td>22</td>
</tr>
<tr>
<td><em>Odocoileus hemionus columbianus</em></td>
<td>50000</td>
<td>35-70</td>
<td>23</td>
</tr>
<tr>
<td><em>Ovis aries</em></td>
<td>75000</td>
<td>46-75</td>
<td>24</td>
</tr>
<tr>
<td><em>Rangifer tarandus</em></td>
<td>102000</td>
<td>200</td>
<td>25</td>
</tr>
<tr>
<td><em>Myotis lucifugus</em></td>
<td>7.9</td>
<td>80</td>
<td>26</td>
</tr>
<tr>
<td><em>Eptesicus fuscus</em></td>
<td>17</td>
<td>115</td>
<td>27</td>
</tr>
<tr>
<td><em>Ailurus fulgens</em></td>
<td>4000</td>
<td>200</td>
<td>28</td>
</tr>
<tr>
<td><em>Homo sapiens</em></td>
<td>59000</td>
<td>28(^a) (40-48)</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25(^a) (36-42)</td>
<td></td>
</tr>
<tr>
<td><em>Antechinus stuartii</em></td>
<td>28</td>
<td>74</td>
<td>30</td>
</tr>
<tr>
<td><em>Caluromys philander</em></td>
<td>300</td>
<td>78</td>
<td>32</td>
</tr>
<tr>
<td><em>Macropus eugenii</em></td>
<td>5000</td>
<td>74</td>
<td>33</td>
</tr>
<tr>
<td><em>Macropus rufogriseus</em></td>
<td>12000</td>
<td>72</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) average, not peak, intake. Values in brackets are estimated peak intakes based on an average of 60-70% of peak (Oftedal, 1985).

1 Glazier (1985)                   2 Millar (1979)                   3 Stebbins (1977)
7 Lochmiller et al. (1982)        8 Weiner (1987a)                 9 Smith and McManus (1975)
10 Myrcha et al. (1969)           11 Wang (1925)                  12 Slonaker (1925)
16 Kenagy et al. (1990)           17 Haver (1979)                 18 Melcher et al. (1989)
The increases in intake during gestation and lactation could be expected to increase the rate of passage of food through the gut (Warner, 1981) and hence decrease the digestibility of the diet due to a decrease in the time for digestion and absorption (Blaxter et al., 1956; Grovum and Hecker, 1973; Grovum and Williams, 1973a; Parra, 1978). However, digestibility is not reduced during reproduction, but maintained or improved in sheep (Graham, 1964; Hadjipieris and Holmes, 1966), Sigmodon hispidus (Randolph et al., 1977; Mattingly and McLure, 1982), mice (Myrcha et al., 1969; Smith and McManus, 1975; Studier, 1979), rabbits (Partridge et al., 1983), voles (Kaczmarski, 1966; Migula, 1969), hamsters (Weiner, 1987a) and porcupine (Farrell and Christian, 1987). This is probably due to the ability of many mammals to increase gut capacity (Myrcha, 1964, 1965; Gross et al., 1985; Green and Millar, 1987; Bozinovic et al., 1990; Loeb et al., 1991; Foley and Cork, 1992; Hammond and Diamond, 1992), and/or the absorptive ability of the gut (Karasov and Diamond, 1983; Hammond and Diamond, 1992), in response to increased energetic requirements, which counteracts the depressive effects of increased intake on retention time in the gut.

In some cases the digestibility of the diet may even rise during reproduction, despite large increases in intake. In Phodopus sungorus, digestibility of the diet rose towards the end of gestation (Weiner, 1987a). Red deer hinds choose better quality pasture and food when reproductive demands are high (Clutton-Brock et al., 1982; Loudon and Kay, 1984), thus behaviourally raising the digestibility of their diet and also presumably their ability to produce milk, as Loudon et al. (1984) found that milk yield of red deer hinds on good pasture was twice that of hinds on poor pastures. Female dolphins, Stenella attenuata, also show a dietary shift during lactation, consuming a greater proportion of fish and less of squid than pregnant females (Bernard and Hohn, 1989). Dietary quality also affects milk composition and production in domestic ruminants (Thomas and Martin, 1988).

Several authors have suggested that maximal rates of energy expenditure in mammals (Peterson et al., 1990) are limited by the ability for intake and assimilation of food energy, and that in many species intake by lactating females is close to some theoretical maximum of energy use (Kirkwood, 1983; Kenagy et al., 1989b; Weiner, 1987a, b, 1992; Hammond and Diamond, 1992). Others have suggested that there is a limit to maximal expenditure (i.e. metabolic scope) that limits energy use by animals (Drent and Daan, 1980). Whether or not the energy requirements of female mammals approach some physiological maximum during lactation, the energy requirements of lactating females are generally the greatest of any members of the population.
(Kenagy et al. 1990), making it likely that their requirements set the lower level of habitat quality that can support a reproducing population.

In summary, energetic demands of reproductive processes in general, especially lactation and gestation, are met mainly by an increase in intake of energy as food by the reproductive female, usually mainly at the time of reproduction. This increase in energy required from the environment and its magnitude, especially at peak, is a major determinant of the habitat and food quality required by any self perpetuating population. Requirements for reproduction must therefore be of concern not only in theoretical aspects of biology such as evolutionary or behavioural ecology but also for the practical conservation and management of a species.

1.4.2 Metabolic changes (Strategy 2).

Production of foetal tissue is 11-14% efficient in domestic ruminants (ARC, 1980), so 86 to 89% of the extra metabolizable energy intake by the mother is towards the development and maintenance of maternal tissue associated with gestation (e.g. placenta, mammary gland) and maintenance of the foetus. Production of milk is 60-90% efficient (Thomson et al., 1970; ARC, 1980, Partridge et al., 1986); more efficient than gestation largely because the maintenance of the young is not included in the maternal energy budget. Therefore the production of $598 M_m^{0.65}$ kJ.d$^{-1}$ of milk energy (Table 1.1, Oftedal, 1984) should be accompanied by $66-399 M_m^{0.65}$ kJ.d$^{-1}$ of maternal energy use in production of that milk. The large increases in food energy intake also require energy for acquiring as well as processing that food-specific dynamic action (SDA, 15% of metabolizable energy intake, Kleiber, 1975). Nevertheless, despite the energy required to support production, maternal metabolic rate may not be elevated during gestation or lactation.

High BMR has been postulated to be advantageous for reproduction (McNab, 1980, 1986). Thompson and Nicoll (1986) and Nicoll and Thompson (1987) suggested that species with a low basal metabolic rate (BMR) raise their metabolism during reproduction (resting metabolic rate, RMR) closer to the predicted eutherian average, while in species with high BMR the RMR during reproduction is close to non-reproductive BMR. In Monodelphis domestica, Elephantulus rufescens, Echinops telfairii (Thompson and Nicoll, 1986), Thrichomys apereoides, Hemicentetes semispinosus (Nicoll and Thompson, 1987) and Didelphis virgiana (Fleming et al., 1981), species with low BMR, increases in RMR ranged from 14-44% during gestation (with one huge increase of 266% in Hemicentetes) and from 15-132% during lactation. However, in Aepyprymnus rufescens, a marsupial with BMR 11 to 20% below the predicted eutherian average, RMR of lactating females remained at
non-reproductive levels (Wallis and Farrell, 1992). This suggests that a large rise in RMR in species with low BMR is not a necessary part of reproduction.

RMR during reproduction has also been reported to decrease or remain at non-reproductive levels in humans (Prentice and Whitehead, 1987), white-tailed deer (Moen, 1978) and a number of rodent species (Trojan and Wojciechowska, 1967; Randolph et al., 1977; Studier, 1979; Mattingly and McLure, 1982; McLure, 1987; Weiner, 1987a; Nicoll and Thompson, 1987). For maternal metabolic rate to decrease or remain constant despite the additional energy expenditure required for production and SDA, there must be some form of compensation in the maternal energy budget, i.e. there must be a reduction in some other component of metabolism.

Some mammals reduce maternal energy metabolism through a reduction in expenditure for thermoregulation or activity. For instance, energy flux in free-ranging reproductive bats has been found to increase less than expected during gestation and lactation, probably due to maternal heterothermy during reproduction (Speakman and Racey, 1987; Racey and Speakman, 1987). The incidence of torpor decreases at the end of gestation and then becomes important again in lactation, suggesting that heterothermy may be incompatible with rapid foetal growth at the end of gestation (Speakman and Racey, 1987). Compensation of this sort may be necessary for bats to minimize the extremely high energetic demands of extra foraging (Kurta et al., 1989, 1990). Nicoll and Thompson (1987) also suggested that part of the increase in RMR observed in reproductive females of species with low BMR may be due to a decline in their degree of heterothermy. Compensation in maternal respiration may also be achieved by a reduction of activity, as seen in laboratory rats (Slonaker, 1925; Wang, 1925), Sigmodon hispidus (Randolph et al., 1977) and in reduction of home-range in red deer (Clutton-Brock et al., 1982).

During gestation and lactation reproductive tissues (i.e. placenta, mammary gland) have special nutritional requirements. The increases in mammary requirements result in significant increases in cardiac output and mammary blood flow (Hanwell and Peaker, 1977), hepatic gluconeogenesis, uptake of minerals (see Vernon, 1988) and lipolysis in adipose tissue (Vernon and Flint, 1984). The production of lactose for export in milk requires 70% of the glucose production in lactating ewes (Graham, 1964). These changes in maternal metabolism are vital to the export of energy in milk and affect the specific maternal nutrient requirements.

Changes in maternal metabolism increase the efficiency of some metabolic pathways. Lipolysis effectively increases during lactation due to hormonal suppression of esterification of triacyl-glycerides in adipose tissue for deposition as fat (Vernon and
Flint, 1984). Suppression of the activity of this enzyme results in less cycling of lipolysis and re-esterification in the adipose tissue, and hence both greater net lipolysis and more efficient export of lipids as milk (Vernon and Flint, 1984). Similarly, the thermogenic activity of brown adipose tissue is suppressed during lactation, possibly by the hormone prolactin (Chan and Swaminathan, 1990), reducing maternal metabolic rate. Reproducing female rats, mice and hamsters convert a greater proportion of metabolizable energy to production than non-reproductives largely due to the suppression of thermogenesis in brown adipose tissue (Trayhurn et al., 1982; Naismith and Brookes, 1983; Roberts and Coward, 1984; Wade et al., 1986). Urinary nitrogen excretion is reduced during lactation in sheep (Graham, 1964), which may be due to greater recycling of urea to the gut.

Parturition itself is relatively brief and the energy requirements largely unknown. Graham (1964) noted extreme increases in respiration of gestating ewes within 24 hours of parturition. However, the brevity of parturition in comparison with other reproductive events mean that its requirements are likely to be relatively low (Gittleman and Thompson, 1988).

In summary, changes in maternal respiration occur in many mammals during reproduction, and failure to consider them may cause serious error in the estimation of reproductive requirements. Production by reproductive females requires expenditure of metabolizable energy, but in some species there is some form of energetic compensation, such as reduction of expenditure for thermoregulation or activity.

**1.4.3 Changes in maternal energy stores (Strategy 3).**

Maternal energy stores can be very important in meeting the daily requirements for reproduction, especially during lactation, although the degree of reliance on stored energy ranges from none to total (Wang, 1925; Hytten and Thomson, 1968; Eisley, 1971; Randolph et al., 1977; Mattingly and McLure, 1982; Partridge et al., 1983, 1986; Sadleir, 1984; McLure, 1987; Kenagy, 1987; Millar, 1987; Tyler 1987; Weiner, 1987a; Kenagy et al., 1989b; Kurta et al., 1989). These stores are often laid down during early gestation. This hyperphagia is due to the projected future demands of lactation rather than the current demands of gestation (Figure 1.1). In *Sigmodon hispidus*, increases in maternal stores accounted for 25% of the increased intake during gestation and subsequently supplied nearly 20% of the energetic demand of lactation (Randolph et al., 1977). Weiner (1987a) reported a similar pattern for *Phodopus sungorus*. Randolph et al. (1977) calculated that in the absence of storage the increased food energy intake required for gestation would be 16% (c.f. 25% in Table 1.2) and 88% for lactation (c.f. 66% in Table 1.2). *Neotoma floridana* stored
even larger amounts of energy during gestation (85% of the increased intake), which supplied 29% of lactational demands (McLure, 1987). Partridge et al. (1986) calculated that in rabbits the metabolic energy expenditure required to store and later recover that energy results in a surprisingly small decrease in overall efficiency of energy utilization. This is due to the high efficiency of utilization of stores in milk production (94% vs 74% for dietary metabolic energy). Thus storage of fat is an efficient method of spreading out the demands of reproduction over time and minimizing increases in daily food intake at times of peak demand.

Among smaller mammals the storage of energy during early gestation, or utilization during late gestation, is less important than in large mammals, although it does occur (see Sigmodon, Neotoma and Phodopus above). Glazier (1985) concluded that little or no energy was stored in any of five species of Peromyscus he investigated, while Millar (1987) suggested that stores act as a buffer against short term shortfall in most small species rather than as a systematic part of the financing of reproductive output. He also suggested a relationship between energy availability and use of reserves, with those species having poor food resources relying most heavily on reserves. Bats store little energy during gestation, less than 1 day's lactational demand in Myotis lucifugus (Kurta et al., 1989). This is probably due to the high energy costs of carrying that mass increase during flight (Kurta et al., 1989).

Figure 1.1 The pattern of energy intake and storage during reproduction in the Djungarian hamster (from Weiner, 1987a)
Among larger mammals, maternal stores are even more important in spreading the demands of reproduction over a longer period, than in small mammals. Ungulates such as Peary caribou (Thomas, 1982), Svalbard reindeer (Tyler, 1987) and domestic ruminants (Vernon and Flint, 1984; Vernon, 1988) rely on maternal stores in late gestation and lactation. Phocid pinnipeds and "denning" bears rely exclusively on stored energy for the maintenance of lactation (Fedak and Anderson, 1982; Bonner, 1984; Ortiz et al., 1984; Costa et al., 1986; Ramsay and Dunbrack, 1986; Anderson and Fedak, 1987; Watts and Hansen, 1987; Oftedal et al., 1987; Bowen et al., 1992).

It is the depletion of stores that could have been used in future reproduction and thus possibly reducing subsequent fecundity and reproductive success (Thomas, 1982; Loudon, 1987b) that is the most likely mechanism linking reproductive "effort" and "cost" in mammals that rely on energy stores in reproduction (Clutton-Brock et al., 1982). However, in mammals that rely entirely or largely on current intake to fund reproduction the above mechanism is unlikely, suggesting that "cost" and "effort" may not be closely linked.

1.5 MARSUPIALS AND EUTHERIANS: A TALE OF TWO STRATEGIES.

The debate on the relative superiority of eutherians and marsupials was originally fuelled by the apparently primitive nature of marsupial thermoregulation and reproduction with its heavy reliance on lactation, together with the apparent replacement of marsupials by eutherian competitors (Lillegraven, 1975). Much of this supposed inferiority was thought to be due to the inability of marsupials to sustain the hormonal and nutritional conditions required to sustain prolonged gestation. While a detailed analysis of physiological differences between the two reproductive strategies is beyond the scope of this thesis, it is also unnecessary as marsupials probably possess the potential for extended gestation (Tyndale-Biscoe and Janssens, 1988). More recent analysis has suggested that the different reproductive patterns are better viewed as alternative viable strategies (Hayssen et al., 1985; Lee and Cockburn, 1985; Tyndale-Biscoe and Janssens, 1988).

The major difference between eutherians and marsupials is the relative degree of dependence on gestation and lactation for transfer of energy from mother to young. Lee and Cockburn (1985) showed that while production during gestation is far lower in marsupials than eutherians (Equation 1.11), the mass of young weaned is similar in ecologically similar marsupials and eutherians. Therefore marsupials rely on lactation
for a much greater proportion of the energy required and a greater degree of development.

Lactation in marsupials can be divided into three phases corresponding to development of the young (Tyndale-Biscoe and Janssens, 1988). Phase 1 is the development of the capacity for milk synthesis during gestation, as in eutherians. Phase 2 is the period when the young is totally dependent on milk, divided into 2 stages; a) when the young is continuously attached to the teat (there is no equivalent in eutherians) and b) when the young is not continuously attached. Phase 3 is the period after first pouch exit to the end of weaning, equivalent to lactation in eutherians.

Due to the greater degree of development supported by lactation in marsupials than eutherians there are differences in the way the mammary gland develops and far more profound changes in milk composition over the lactation (see Green, 1984; Green and Merchant, 1988).

Dove and Cork (1989) showed that the form of the equation describing the temporal pattern of metabolic energy requirements of the mother to support reproduction was similar between Macropus eugenii and dairy cattle. They suggested that stages 1 and 2 (Tyndale-Biscoe and Janssens, 1988) of marsupial lactation (from conception to pouch exit) were energetically equivalent to gestation in eutherians, and that phase 3 of marsupial lactation was energetically equivalent to eutherian lactation. Similar comparisons have also been made on developmental criteria and Russell (1982a) has suggested that the time of development of endothermy in marsupial pouch young was more closely equivalent to birth in eutherians.

McNab (1980, 1986, 1987) argued that the reproductive rate of mammals was causally correlated with BMR. He suggested that high metabolic rates allowed greater rates of biosynthesis, and so higher reproductive rates, than did low metabolic rates. This argument relied on the assumption of unlimited resources available to the reproductive individual (McNab, 1980), a condition that is unlikely to be the norm. Marsupials have BMR of about 70 % of the predicted level for eutherians (Dawson and Hulbert, 1970; Kinnear and Shield, 1975). This led McNab (1986, 1987) to suggest that low BMR constrained marsupials to a limited set of ecological niches by the mechanism of low reproductive rates. He also suggested that marsupials could co-exist with eutherians only if they consumed diets that were correlated with low rates of metabolism in eutherians, otherwise the higher reproductive rates of eutherians would competitively displace the marsupials. More recent analyses show that although both metabolic and reproductive rates are correlated with body size in
mammals (Fenchel, 1974; Henneman, 1983; Elgar and Harvey, 1987; Nagy, 1987; McNab, 1988), when variation due to body size is controlled the residual variation in BMR is not correlated with reproductive rate inter- or intraspecifically (Ross, 1988; Harvey et al., 1991; Hayes et al., 1992), or with rates of biosynthesis (Derting and McLure, 1989). On the contrary, when food was restricted, cotton rats with high (induced) BMR suffered retardation of growth (Derting, 1989). Thompson and Nicoll (1986) and Nicoll and Thompson (1987) found that some marsupials (and eutherians with low BMR) increased their rate of metabolism during reproduction to or above the predicted eutherian level, while eutherians with high BMR did not increase RMR during reproduction. They argued that this suggested that the optimal level of metabolic rate for reproduction was close to the eutherian average BMR. Presumably the elevation in RMR that they observed was due to the demands of milk synthesis, while the high BMR species compensated in some way for that energy expenditure.

There is no reason, theoretical or empirical, to suggest that marsupials are reproductively inferior to eutherians due to lower rates of metabolism.

Parker (1977), Low (1978) and Hayssen et al. (1985) have suggested that marsupial reproduction should be regarded as adapted to environmental uncertainty, easily allowing termination of reproduction at birth with little energy cost to the mother because of the small size of the neonate. This approach has been criticized by Russell (1982b), Morton et al. (1982) and Lee and Cockburn (1985) as being representative only of macropods among the marsupials, while eutherians also can terminate reproduction during gestation.

The efficiency of maternal production is greater in lactation than gestation (ARC, 1980), due to the maintenance of the placenta and foetus during gestation. However, Glazier (1990a,b) found that efficiency of production of offspring biomass was greater during gestation than lactation in rodents, probably due to the energy requirements for maintenance (especially thermoregulation) of the young during lactation which is not considered in the above maternal production efficiency. He suggested that the greater efficiency of gestation would result in more efficient reproduction in precocial than altricial rodents. It is surprising then that Cork and Dove (1989) found that total requirements for reproduction in a marsupial (highly altricial in terms of energy content of the young) were similar to those in some eutherians and that the efficiency of utilization of maternal metabolic energy for growth of offspring was similar to that in eutherian gestation (Cork, 1991). It is likely that low maintenance requirements of the young marsupial (associated with low adult BMR) and low thermoregulatory expenditure (due to the thermal environment of the
pouch) allowed it to allocate a greater proportion of ingested energy to growth than a similar eutherian young during lactation.

The duration of the female's energetic input to the young (time from conception to weaning) is longer in marsupials than eutherians, except in the middle size category (1-3.5 kg, S. Thompson, 1987) while the size of the litter at weaning is about the same in the two groups. If the efficiency of utilization of maternal energy for offspring production is similar, this suggests a lower rate of energy expenditure in marsupials and hence lower daily requirements for reproduction. Cork and Dove (1989) compared the time course of energetic requirements for reproduction in a marsupial, *Macropus eugenii*, with that for two domestic ruminants, sheep and cattle. They found that the total metabolizable energy requirement for reproduction was a similar proportion of female mass in each species, but that in *M. eugenii* the energy requirements were spread over a longer period than in sheep or cattle, with a much lower peak requirement. They interpreted the pattern in *M. eugenii* as an adaptation of a small herbivore to conditions of low energy availability.

In summary, the modes of reproduction displayed by marsupials and eutherians are best regarded as alternative strategies. In this way the ecological consequences of differing strategies of allocation of energy to reproduction can be considered.
CHAPTER TWO

THE KOALA AND OTHER MAMMALIAN ARBOREAL FOLIVORES: ON BEING A KOALA

2.1 THE PROBLEMS OF BEING AN ARBOREAL FOLIVORE

2.1.1 Introduction to arboreal folivores

Mammalian arboreal folivores are those species adapted to living in trees which also eat foliage. Eisenberg (1978) defined arboreal folivores as those species that spend over 50% of their foraging time in trees and whose diet contains at least 30-40% tree leaves, buds, flowers and shoots, although there is a continuum of degrees of both arboreality and folivory. Arboreal folivores generally also nest in trees, possess structural modifications for arboreality and have digestive tracts showing modification (enlargement and/or lengthening) correlated with their diet of foliage (Eisenberg, 1978). In this thesis, foliage denotes the leaves of trees unless otherwise indicated. Of the total of 1015 mammalian genera only 42 (4%), from 7 of the 14 orders, contain arboreal folivorous species, compared with the 233 genera (23 %) containing herbivorous species (Eisenberg, 1978). Thus, arboreal folivory is a relatively unusual specialization among mammals. The koala, *Phascolarctos cinereus*, is one of the most arboreal and most folivorous mammal species (Eisenberg, 1978). Other highly folivorous and highly arboreal species are within the genera *Petauroides* and *Pseudocheirus* (Marsupialia), *Indri*, *Presbytis*, *Lepilemur*, *Colobus* and *Nasalis* (Primates), *Cynocephalus* (Dermoptera), *Bradypus* (Edentata) and *Dendrohyrax* (Hyracoidea) (Eisenberg, 1978).

Arboreal folivores are middle-sized mammals, from 0.7 to 20 kg body weight and 100-1000 mm head and body length (Eisenberg, 1978). There seem to be physical and physiological constraints to both their upper and lower size limits. There are no arboreal folivores of greater than 15 kg mass. Presumably this is related to either the physical strength of the terminal branches of trees where the foliage is found (Grand, 1978) or the energetic expenditure required to move a large animal vertically (Grand, 1978; Degabriele and Dawson, 1979). The opposing constraint is related to digestive capacity. Because the energetic requirements of mammals increase logarithmically with increasing mass (Kleiber, 1975; McNab, 1980; Nagy, 1987), the mass-specific metabolic requirements of small mammals are greater than those of large mammals. However, Parra (1978) found that the gut capacity of herbivores varied almost linearly with body mass. Parra (1978) and Demment and Van Soest (1985) used these
relationships to calculate a theoretical lower size limit of about 10 kg for foregut-fermenting herbivorous mammals. Below this size, as many arboreal folivores are, the reliance on fermentative digestion must decrease, so there is increasing reliance on strategies such as selectivity in food choice and/or digestive specializations to meet energy requirements (Parra, 1978; Demment and Van Soest, 1985; Cork and Foley, 1991). The question of limitation to fermentative digestion is dealt with in Section 2.1.2.3.

2.1.2 The nutritional problems of folivory

The nutritional problems associated with folivory include poor nutritional quality of foliage, as well as allometric constraints on the utilization of poor quality diets by mammals in the arboreal folivore size range (Section 2.1.2.3). The overall nutritional quality of foliage is determined not only by concentrations of primary nutrients (Section 2.1.2.1), but also of defensive compounds (Section 2.1.2.2).

2.1.2.1 Primary nutrients

Strictly arboreal mammals can have fruit, flowers, seeds and insect foods available to them in addition to foliage, though the availability of these non-foliage foods is patchy in time and space (A. Hladik, 1978). Foliage generally contains more protein than fruits and a similar amount to seeds, but considerably less than insects (Table 2.1). Foliage also contains high levels of structural carbohydrates (fibre) and is more highly lignified than fruits or insects, so the digestibility and availability of the nutrients in foliage are likely to be less (see below).

In comparison with the foods available to less strictly arboreal herbivores (i.e. grasses), the foliage of trees is similar or lower in fibre and protein content but the fibre is more highly lignified. Several studies show that the fibre of tree foliage is less digestible by ruminants than that of herbage or grasses (Short et al., 1974; Robbins et al., 1975, 1987, 1991; Wilson, 1977). In herbage, digestibility of cell walls is correlated with their degree of lignification (Van Soest, 1977, 1982; Robbins et al., 1987), so the high levels of lignin in foliage may reduce its digestibility (Cork et al., 1983; Robbins et al., 1987).

Young foliage differs from mature foliage in having generally lower fibre levels and a lesser degree of lignification of that fibre (Table 2.1). This makes the younger foliage potentially more digestible. In the rainforest trees evaluated by most of the studies in Table 2.1, protein levels in young foliage were greater than in mature foliage; this is
generally but not always the case for *Eucalyptus* foliage (Cork, 1984; Cork and Pahl, 1984; Foley, 1987; Foley and Hume, 1987c; Kavanagh and Lambert, 1990).

**Table 2.1 Composition of foods available to arboreal mammals, as well as grasses.**

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Water^a</th>
<th>Protein</th>
<th>Fibre^b</th>
<th>Lignin</th>
<th>Lipid</th>
<th>Energy^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bark</td>
<td></td>
<td>36</td>
<td>76</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Non-eucalypt foliage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>54-58^4,8,16</td>
<td>7-36^7,8,11,12</td>
<td>15-43^7,8</td>
<td>9-28^8,19</td>
<td>1-11^11,12,19</td>
<td>18-22^4</td>
</tr>
<tr>
<td>Mature</td>
<td>46-69^4,8,13</td>
<td>5-32^7-13,15</td>
<td>27-74^7,8,10,13,15</td>
<td>12-47^8,10,13,15,19</td>
<td>1-11^10-13,19</td>
<td>17-23^4,10,13</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>53-65^1,2</td>
<td>8-11^1,2</td>
<td>18-20^1,2</td>
<td>6-9^1,2</td>
<td>8-15^1,2</td>
<td>20-22^1,2</td>
</tr>
<tr>
<td>Mature</td>
<td>48-56^1-3</td>
<td>7-12^1-3</td>
<td>29-51^1-3</td>
<td>10-20^1-3</td>
<td>11-19^1-3</td>
<td>23-25^1-3</td>
</tr>
<tr>
<td>Flowers</td>
<td>65-86^4,5,16</td>
<td>4-25^16</td>
<td>-</td>
<td>-</td>
<td>1-4^16</td>
<td>18^4</td>
</tr>
<tr>
<td>Fruit</td>
<td>56-87^4,5,16</td>
<td>3-10^5,11,16</td>
<td>8-22^14</td>
<td>0.1-3^14</td>
<td>1-21^5,11,16</td>
<td>17-24^4,5</td>
</tr>
<tr>
<td>Seeds</td>
<td>18-89^16</td>
<td>4-15^16,17</td>
<td>3-47^174</td>
<td>-</td>
<td>1-73^16,17</td>
<td>17-30^17</td>
</tr>
<tr>
<td>Grasses</td>
<td>30-90^20</td>
<td>7-18^18</td>
<td>40-80^18</td>
<td>3-15^18</td>
<td>3-10^22</td>
<td>-</td>
</tr>
<tr>
<td>Insects</td>
<td>63-70^21,23</td>
<td>56-73^16,21,23</td>
<td>15^23</td>
<td>-</td>
<td>15-22^21,23</td>
<td>4-26^11,16,21,23</td>
</tr>
</tbody>
</table>

^a % fresh weight  
^b Neutral detergent fibre (Goering and Van Soest, 1970)  
^c kJ.g\(^{-1}\)  
^d Acid detergent fibre

Lipid levels in *Eucalyptus* are high compared with grasses; however, much of this lipid component can be essential oils (Foley, 1987; Foley *et al.*, 1987) and waxes (Horn *et al.*, 1964) involved in plant defence and consequently unavailable or deleterious to the folivore (Foley, 1987, 1992; Foley *et al.*, 1987).
Mineral content of foliage varies between species of tree as well as temporally within the species (A. Hladik, 1978; Nagy and Milton, 1979b; Baranga, 1983). Nagy and Milton (1979b) suggested that mineral deficiencies in foliage preferred by howler monkeys are corrected by selection and consumption of food items high in those minerals, explaining part of the dietary diversity of howler monkeys and possibly other folivorous primates (A. Hladik, 1978). In *Eucalyptus* forests, density of arboreal folivores is correlated with an index of foliage composition that is strongly correlated with concentrations of potassium and phosphorus (as well as nitrogen) (Braithwaite *et al.*, 1983), although potassium and phosphorus concentrations are also negatively correlated with levels of phenolic compounds in *Eucalyptus* foliage (Cork, 1992).

The nutritional quality of foliage depends not only on concentrations of primary nutrients, but also the negative aspects of leaf composition, plant chemical defenses or secondary compounds.

### 2.1.2.2 Plant defence

"The plant world is not coloured green; it is coloured morphine, caffeine, tannin, phenol, terpene, canavanine, latex, phytohaemagglutinin, oxalic acid, saponin, L-dopa, etc."

Janzen (1978)

Plants are not undefended food, ripe for the eating. On the contrary, they contain a wide variety of chemicals, known as secondary compounds or allelochemicals (Whittaker and Feeny, 1971), which are postulated to have evolved as defensive mechanisms against predation by herbivores (Fraenkel, 1959; Whittaker and Feeny, 1971; Swain, 1977; Harborne, 1988), although some allelochemicals may have other functions; such as resistance to micro-organisms (Swain, 1979; Zucker, 1983), or in competitive interactions with other plants- allelopathy (Bernays *et al.*, 1989). Fibre and lignin may be considered as allelochemicals despite their primary functions and evolutionary origin (Rhoades, 1979), as they present "...substantial barriers to evolution of small mammals into folivore niches" (Cork and Foley, 1991). The evolutionary relationship of herbivores and plant defenses have involved numerous adaptations and counter-adaptations, and in some instances may represent co-evolution of plants and their herbivores (Ehrlich and Raven, 1964; Berenbaum and Feeny, 1981; Berenbaum and Zangerl, 1988; Harborne, 1988).
There have been several attempts to formulate a general theoretical framework explaining the variation in types and quantity of plant defenses. The plant "apparency" theory (Feeny, 1976; Rhoades and Cates, 1976; Rhoades, 1979) predicted that "apparent" plants (plants that are common, widespread and predictable, usually late successional) should be defended by quantitative, digestibility-reducing, carbon-based allelochemicals such as phenolics (including tannins), lignin, and fibre, while "non-apparent" plants (ephemeral or uncommon, often early successional) should be defended by more qualitative, acutely toxic allelochemicals often containing nitrogen.

Notable exceptions between predictions of the plant "apparency" theory and the observed patterns of plant defense and effects on herbivores (Fox and Macauley, 1977; Bernays, 1981; Coley, 1983; Faeth, 1985) led to the formulation of a theory based on the interaction of plant physiology and abiotic factors. The resource-availability hypothesis (Bryant et al., 1983; 1985a, b; Coley et al., 1985) predicts that the observed patterns of plant defense are associated with the balance of carbon and nitrogen available to the plant and the effects of that balance on metabolic pathways in the plant. More specifically, the resource-availability hypothesis predicts that if carbon is in excess and growth is limited by low nitrogen availability, excess carbon from photosynthesis will be allocated to the metabolic pathways producing carbon-based allelochemicals, while those with higher nitrogen availability will be able to allocate more carbon to growth and grow more quickly. Under conditions of low carbon and high nitrogen availability (such as shaded but fertilized plants), nitrogen in excess of the requirements of growth may be diverted into pathways producing nitrogen-based allelochemicals. There have been many empirical tests of predictions of the resource-availability hypothesis. Shading or enhancement of the nitrogen status of plants (by fertilization) increases concentrations of nitrogen-based allelochemicals (Mattson, 1980; Van Horne et al., 1988; Herms and Mattson, 1992) and decreases carbon-based defenses (Bryant, 1987; Bryant et al., 1987a,b; Mole et al., 1988; Glyphis and Puttick, 1989; Waterman and Mole, 1989). However, the results of some studies contradict predictions of the resource availability hypothesis; fertilization can increase levels of carbon-based allelochemicals (Clark and Menary, 1980; Lincoln and Mooney, 1984; Muzika et al., 1989).

The growth-differentiation balance hypothesis (Tuomi et al., 1990; Herms and Mattson, 1992), which makes similar predictions to those of the resource-availability hypothesis (Herms and Mattson, 1992), has been proposed to explain some of the inconsistencies of the resource-availability hypothesis. The growth-differentiation balance hypothesis proposes that there is a physiological trade-off between growth
and defense, assuming that there are constraints on them occurring concurrently in the same cells (Herms and Mattson, 1992).

Arboreal folivores are more likely to encounter digestibility-reducing compounds such as tannins, lignin and other phenolics more than acutely toxic compounds. They are also more likely to encounter higher levels of these chemicals than non-folivores, although folivores in tropical forests potentially encounter a wider range of allelochemicals than those in temperate forests (Cork and Foley, 1991). Trees of the genus *Eucalyptus* dominate Australia's forests (Pryor and Johnson, 1971) and grow on soils that are generally low in nutrients (Attiwill and Leeper, 1987). Thus, both the plant "apparency" theory and resource-availability hypothesis predict the observed high levels of carbon-based defenses in *Eucalyptus* foliage, especially fibre, lignin, phenolics and essential oils (Fox and Macauley, 1977; Southwell, 1978; Cork, 1984; Cork, 1992), and the general lack of nitrogen-based defenses, although *Eucalyptus* foliage has been reported to contain cyanogens (Finnemore et al., 1935). Woody plants in general contain higher concentrations of phenolics (including tannins) than do herbaceous plants (Rhoades and Cates, 1979; Swain, 1979; Bernays et al., 1989), as phenolics and lignin are both products of the shikimic acid biosynthetic pathway (Swain, 1979). Thus, I will concentrate on the carbon-based defenses, especially the phenolics and essential oils found in *Eucalyptus* foliage.

The effects of plant allelochemicals on herbivores have been reviewed several times (Freeland and Janzen, 1974; Feeny, 1976; Rhoades and Cates, 1976; Mole and Waterman, 1987; Harborne, 1988) and range from stimulation of feeding by insects (Harborne, 1988) to sudden death (Janzen, 1978). The toxicity of an allelochemical depends on the balance of nutrient gain from the food item and the cost or loss due to the accompanying allelochemicals; hence their description as anti-nutrients (Janzen, 1978), and also depends on the characteristics of both the allelochemical and the herbivore (Freeland and Janzen, 1974; Janzen, 1978; Robbins et al., 1991).

Fibre and lignin levels are high in foliage, especially *Eucalyptus* foliage (Table 2.1). Fibre acts as a plant defense by presenting a difficult and time-consuming bulk to digest (Parra, 1978; Demment and Van Soest, 1985; Duncan et al., 1990; Illius and Gordon, 1992, see Section 2.1.2.3). Diets high in fibre are low in rapidly available energy because the energy and other nutrients in fibre can only be digested in vertebrates by fermenting gut flora (Van Soest, 1982). Rates of fermentation of *Eucalyptus* foliage, measured *in vitro*, in the hindgut of the koala, greater glider and brushtail possum are low compared with fermentation rates in the hindgut of other
mammals, probably due to the high level of lignification of *Eucalyptus* fibre (Cork and Foley, 1991).

The low protein levels found in foliage can also be considered a plant defence strategy (Fox and Macauley, 1977; Hladik, 1977; McNeil and Southwood, 1978), as they minimize the value of the foliage to herbivores and increase the ratio of cost due to the effects of allelochemicals to the benefit gained from eating the tissue.

Tannins and other phenolics can make up a large proportion of foliage dry matter, although exact levels can be difficult to determine due to methodological problems, including differing extractibility between and within plant species (Cork and Krockenberger, 1991). Levels in *Eucalyptus* foliage can be from 13 to 25% of dry matter in mature foliage and even greater (6-40%) in young foliage (Cork, 1984; Cork and Pahl, 1984; Cork and Krockenberger, 1991).

Tannins are polyphenolic compounds that form *in vitro* complexes with protein, as well as with other macro-molecules (Mole and Waterman, 1987a, b), and are postulated to reduce digestibility of dietary proteins and activity of digestive enzymes *in vivo* (Feeny, 1976; Swain, 1979), raising the loss of protein in the faeces (Cork, 1986; Foley and Hume, 1987b, c; Robbins *et al*., 1987; Mole and Waterman, 1987a). In arboreal folivores this is a substantial problem due to the already low levels of protein in their food (Table 2.1). Dietary tannins have a range of effects on vertebrate herbivores including reduced food intake and digestibility, low palatability, depression of growth rates and increased loss of faecal nitrogen, although the mechanism of these effects is unclear (Mole and Waterman, 1987a; Robbins *et al*., 1987, 1991; Blytt *et al*., 1988; Hanley *et al*., 1992).

Formation of complexes by tannins depends on pH and ionic strength (Martin *et al*., 1985). Freeland *et al*., (1985a) found that tannins produce erosion of gut walls and a mineral deficiency in mice. This effect was prevented by mineral supplementation, and the authors postulated that cations were used to raise ionic strength in the gut and so avoid the action of the tannin. Similarly, Feeny (1976) suggested that high hindgut pH may reduce the effects of tannins in some insects. Hladik and Gueguen (1974) and Hladik (1977) have suggested that primates may ingest clay soils to counteract the effects of dietary tannins. In some mammals which consume high levels of dietary tannin, proline-rich proteins secreted in the saliva may act to minimize the effects of those tannins on dietary or intestinal proteins by preferentially complexing with the tannins (Robbins *et al*., 1987, 1991; Mole and Waterman, 1987a). This may help to maximize the availability of essential dietary amino acids by minimizing the quantity of tannin available to complex with dietary proteins (Cork and Foley, 1991).
The effect of tannins on the digestion of protein can alter with the proportions of tannins and protein, and can increase digestibility at some of the ratios commonly found in foliage (Mole and Waterman, 1985). There is some evidence that tannins may reduce the effects of other allelochemicals such as alkaloids, saponins and cyanogenic glycosides (Freeland and Janzen, 1974; Freeland et al., 1985b; Goldstein and Spencer, 1985; Martin et al., 1985), and in some cases may increase nitrogen retention in ruminants by protecting dietary protein from microbial degradation in the rumen (Barry and Manley, 1984).

Hydrolysable tannins are broken down in the gut to their component sugar and phenolic moieties. The sugar moiety may be used like other dietary sugars (Cork et al., 1983) but the phenolic group must be detoxified and excreted (Brattsten, 1979), which may disrupt the acid-base balance of the herbivore's blood as well as requiring energy to detoxify and excrete the metabolite (Foley, 1992).

Essential oils, named for their aroma, are a complex mixture of terpenoids (a group of carbon-based compounds) found in foliage. Levels in Eucalyptus foliage vary greatly between species. Southwell (1978) reported from 0 to 3.4 % oils in koala food tree species, and levels can reach 13 % of dry matter (Foley, 1987). The efficiency of utilizing digested energy can be far lower with high terpene (essential oil) diets than low terpene diets due to the excretion of the energy-rich oil metabolites in the urine (Cork et al., 1983; Chilcott and Hume, 1984a; Foley, 1987, 1992).

Several Eucalyptus oil components have anti-bacterial properties (Southwell, 1978). Anti-bacterial activity of essential oils has been noted in foregut fermenters (Nagy et al., 1964; Oh et al., 1967), though prolonged intake of the oils leads to a tolerant microbial population (Oh et al., 1967; Freeland and Janzen, 1974). Toxicity may be due to dissolution in membranes and disruption of membrane function (Haagen-Smit, 1948; Andrews et al., 1980). Detoxification of allelochemicals by such an adapted gut flora could be one of the major functions of foregut fermentation in arboreal folivores (Freeland and Janzen, 1974; Dasilva, 1992). However, hindgut fermentation may be better suited to deal with diets high in essential oils, as most is absorbed in the stomach and small intestine, so little (4-15 %) of the oil reaches the hindgut where the flora are active (Eberhard et al., 1975; Foley et al., 1987).

When allelochemicals such as essential oils or the phenolic portion of hydrolysable tannins and other phenols are absorbed, they are detoxified and excreted (Baudinette et al., 1980; Cork et al., 1983; Foley, 1987, 1992; Foley and Hume, 1987c). Detoxification in the liver is catalysed by the mixed-function oxidase enzyme system (Williams, 1959; Brattsten, 1979). This enzyme system uses NADPH as an energy
source, and glucuronic acid (Williams, 1959), sulphate or glycine (Brattsten, 1979) as conjugates for excretion in the urine for low- or in the bile for high-molecular mass compounds (Hirom et al., 1976). The energy and nutrients required for detoxification and excretion of allelochemicals represent the cost of those allelochemicals to the folivore. Glucuronic acid is often the main conjugate used by marsupial folivores (Baudinette et al., 1980), presumably due to the low levels of nitrogen and sulphate available in Eucalyptus foliage, but recent research has shown that hippuric acid is the major excreted conjugate in ringtail possums, while a large proportion of the ingested allelochemicals are excreted unconjugated (Maclean et al., in prep). Glucuronic acid levels are high in the urine of koalas, brushtail and ringtail possums fed Eucalyptus (Hinks and Bolliger, 1957; Cork et al., 1983). Cork (1981) calculated that 20 % of koalas' basal rate of glucose turnover was used in supplying glucuronic acid for excretion of allelochemicals. However, the high ascorbate content of Eucalyptus foliage may act as a substrate for the synthesis of glucuronide, saving endogenous glucose (Dash et al., 1984; Dash, 1988).

Detoxification and excretion of allelochemicals can also lead to acid/base imbalance in folivores (Foley, 1992). In the greater glider and ringtail possum this leads to urine which is high in ammonium and low in urea, and an increased maintenance nitrogen requirement due to the high urinary loss (Foley and Hume, 1987b; Foley, 1992). High urinary nitrogen loss can be detrimental to the maintenance of nitrogen balance by folivores eating a nitrogen-poor diet. The reduction in urea synthesis may also affect the performance of hindgut fermentation, as endogenous urea recycled to the hindgut is a major source of nitrogen for the bacterial flora (Cork and Foley, 1991).

2.1.2.3 Body size and fibre

As outlined in 2.1.1 arboreal folivores occur in a limited mass range of 0.7-20 kg, the upper size limit set apparently by the physical strength of terminal tree branches and/or the energy requirements of vertical locomotion (Eisenberg, 1978). The lower size limit is most likely set by allometric constraints on the relationship between digestive capacity and energetic requirements.

Fibre (or cell wall) in plant tissue is not susceptible to enzymatic digestion by mammalian alimentary enzymes, and so represents a resource unavailable to mammalian herbivores except through a symbiotic relationship with bacteria and/or protozoa, found in expanded hind- or foregut fermentation chambers (Hume and Warner, 1980; Van Soest, 1982). In most habitats, including forests, the biomass of plant tissue low in fibre is small and patchy; dispersed both spatially and temporally, whereas plant tissues high in fibre make up the great majority of the total biomass
Thus the larger the total energy requirements of an animal, *ie.* the larger it is, the more difficult it will be to find sufficient high quality (low in fibre) food. Most large herbivores are forced toward eating a diet high in fibre, while small herbivores with a high mass-specific metabolic rate must select rapidly-digestible high-quality foods (Demment and Van Soest, 1985; see below).

This distribution of resources was originally postulated to explain the differences in feeding behaviour and migratory patterns over the size range of ungulates (Bell, 1971; Jarman, 1974). All ruminants under 15 kg are "concentrate selectors", selecting low-fibre foods (Hofmann and Stewart, 1972; Jarman, 1974; Demment and Van Soest, 1985), while many large ruminants can be classified as unselective bulk-roughage feeders with a high level of dietary fibre. This general relationship of size and level of fibre intake also holds for macropodid marsupials (Hume, 1982) and primates (Clutton-Brock and Harvey, 1977). Small herbivores also tend to have a relatively smaller mouth, perhaps aiding their selectivity, as in small ungulates (Bell, 1971) and the small marsupial folivore, *Pseudocheirus peregrinus*, which is able to select the lowest fibre portion of foliage, biting around the fibrous midrib (Chilcott, 1982).

The acquisition of energy from food is a function of the intake of food and the extent of digestion and absorption (Demment and Van Soest, 1985). The digestibility of food is a function of the rate of digestion, the time for which it is digested (*ie.* retention time) and the fraction that is potentially digestible (Demment and Van Soest, 1985). It is easy to see then that if maximum energy is to be derived from the fermentation of fibre, then it must be retained for a long period (Van Soest *et al.*, 1983). If it is not, or if it is lignified (as in foliage), then the potential gain from the food is substantially lowered.

The gut capacity of mammalian herbivores, both fore- and hindgut fermenters, is linearly proportional to their mass (Parra, 1978; Chivers and Hladik, 1980; Demment and Van Soest, 1985; Illius and Gordon, 1992), and so the amount of food being digested at any one time is linearly related to mass. The retention time of digesta is also related to mass, but with a fractional exponent of approximately 1/4 (Demment, 1983; Illius and Gordon, 1992).

\[
\text{MRT}_{\text{HG}} = 9.4 M^{0.26} \quad \text{Equation 2.1}
\]

\[
\text{MRT}_{\text{FG}} = 15.3 M^{0.25} \quad \text{Equation 2.2}
\]

where MRT_{HG} and MRT_{FG} are the mean digesta retention times in hindgut and foregut fermenting herbivores respectively in hours and M is the body mass in kg.
However, the rate of fermentation of fibre achieved in gut fermentation chambers seems to be largely independent of body mass (Parra, 1978). So the power (ie rate of energy release) that could be obtained from fermentation of fibre is related linearly to body mass. The high fermentation rates measured in vitro from some small ruminants are largely due to their selection of diets high in readily fermentable components and low in fibre (Demment and Van Soest, 1985).

The energetic requirement for free existence (FMR) in mammals varies non-linearly with body mass (Nagy, 1987).

\[
\text{FMR}_E \ (\text{kJ} \cdot \text{d}^{-1}) = 5.94 \ W(\text{g})^{0.727} \quad \text{Equation 2.3}
\]

\[
\text{FMR}_M \ (\text{kJ} \cdot \text{d}^{-1}) = 6.37 \ W(\text{g})^{0.644} \quad \text{Equation 2.4}
\]

where E and M are eutherians and marsupials respectively.

The fractional exponents of mass mean that while the absolute or total energetic requirements of small animals are less than those of large ones, the mass-specific (ie. kJ.kg^{-1}.d^{-1}) requirements are greater. An average 1 kg eutherian herbivore would require nearly twice the energy per kg per day than that required by a 10 kg herbivore, although its gut capacity remains in the same proportion to its mass. So the rate of energy absorption per kg of gut and contents in the 1 kg herbivore must be more than twice that in the 10 kg herbivore to meet energy requirements, allowing for an efficiency of conversion of digestible to metabolic energy of around 75%.

Parra (1978) concluded that herbivores below 100 kg could not rely wholly on fermentation of a fibrous diet, and that below about 10 kg fermentation must play little part. Demment and Van Soest (1985) developed the model further to predict that the lower limit of mass for which a ruminant fermentative digestion is suitable is about 9 kg, but did not model strategies that do not wholly rely on fermentative digestion and did not consider hindgut fermentation (Cork, in prep). Illius and Gordon (1992) modelled size-related constraints on intake and digestion with a similar approach, but extended the model to include hindgut fermenters. Their model predicted a selection pressure for evolution of large body mass in terrestrial herbivores with an abundant food source and limits to body mass imposed by food limitation. However, for their calculations of energy requirement they used maintenance metabolism (Brody, 1945), lower than the energetic demands of free existence, and therefore overestimated the ability of small herbivores to survive on high-fibre diets. Their model predicted that small mammals (\(\leq 1 \text{ kg}\)) could meet maintenance energy requirements from a diet containing up to 60% (foliage) to 65%
(grasses) fibre (Cork, in prep), although empirical studies of small rodents show that most cannot survive on a diet greater than 40% in fibre content, or 55% for voles (Keys et al., 1970; Keys and Van Soest, 1970; Karasov, 1982; Cork and Kenagy, 1989). When the requirements for free existence (Nagy, 1987), which are higher and more realistic, are used in the model, it predicts that small (< 1 kg) mammals with average digestion and metabolism require diets with less than 40% (foliage) to 45% (grasses) fibre (Cork, in prep).

Cork and Foley (1991) concluded that mammals in the arboreal folivorous size range should avoid highly fibrous plant parts such as mature foliage, and select low-fibre parts high in available energy such as young foliage, fruit, flowers and seeds. Exceptions to this generalization occur in species displaying a range of digestive adaptations that relax the physiological limitations on the acquisition of dietary energy (Cork and Foley, 1991; Foley and Cork, 1992).

2.1.3 Solutions to the problems of folivory

2.1.3.1 Food Selection

"... a sloth could starve to death with a full stomach if it made the wrong choice in the kind of leaf it ingested."

Montgomery and Sunquist (1978)

Because of the low nutritional value of foliage (Section 2.1.2.1), high allelochemical content (Section 2.1.2.2) and the limited body size (Sections 2.1.2.3) of arboreal folivores, food choice by these animals is likely to be of extreme importance. Many studies have examined the food choices of arboreal folivores, especially among the primates, and, while certain trends are evident, the basis for food choice remains unclear.

Food choice is dependent on a number of factors at several levels. First, the degree of folivory is related to body size, with the larger arboreal folivores being the most folivorous (Clutton-Brock and Harvey, 1977; C. Hladik, 1978), as predicted from allometric considerations (Section 2.1.2.3). There are exceptions to this rule; Lepilemur in the primates and Pseudocheirus among the marsupials are more folivorous than expected on the basis of body size (Clutton-Brock and Harvey, 1977; Demment, 1983; Chilcott and Hume, 1984a), although this is probably due to their extraordinary digestive specialisation (Section 2.1.3.3). Small primates tend to be insectivorous-frugivorous, and the medium sized tend to be frugivorous-folivorous (C. Hladik, 1978). Small primates can meet all their nutritional requirements from
insects, supplementing their energy source with fruit, but larger primates cannot
gather sufficient insects as a protein source and so must supplement their diet with
foliar protein (C. Hladik, 1978). Moreover, as foliage is low in protein, and high in
bulk (fibre) it cannot be a concentrated supplement, so large primates must eat large
quantities of foliage to satisfy their protein requirements (C. Hladik, 1978). The
degree of folivory can also be related to sex. In folivorous primates males tend to
spend less time feeding and are relatively more frugivorous than females (Indri indri
Pollock, 1977; Colobus badius Clutton-Brock, 1977). This may relate to lower
protein requirements in the males and to greater female requirements during
reproduction (Clutton-Brock, 1977).

Second, folivores tend to select young rather than mature foliage (Chivers, 1977;
Landsberg, 1987; Kavanagh and Lambert, 1990; Pahl and Hume, 1990). In tropical
and Eucalyptus forests young foliage is associated with high protein and low fibre and
lignin content compared with mature foliage (Table 2.1). In most of these studies,
 inclusion of the young leaves as a major part of the diet probably indicated selection
for them, as the young leaves and shoots were much less available than mature foliage
(A. Hladik, 1978; C. Hladik, 1978). Other ephemeral resources such as flowers and
seeds can also be important in the diets of arboreal folivores (A. Hladik, 1978; McKey
et al., 1981).

Selection for young leaves could indicate avoidance of the higher fibre in mature
foliage and/or selection for the higher protein content, although it could also be
related to allelochemical content. Arboreal folivores have generally been reported to
select against fibre (A. Hladik, 1978; Milton, 1979; Oates et al., 1980; Choo et al.,
1981; McKey et al., 1981; Oates et al., 1990; Procter-Gray, 1985 and Goudberg, 1990
McKey et al., 1981; Baranga, 1983). Young foliage has also been perceived as low in
allelochemicals such as phenolics (Clutton-Brock, 1977), although this is not always
Phenolic levels are confused by apparently differing extractability between young and
mature foliage (Bate-Smith, 1973).

Montgomery and Sunquist (1978) demonstrated that the in situ rate of digestion of
foliage preferred by three-toed sloths, Bradypus infuscatus, was faster than that of
rarely or non-selected foliage, and that young foliage was digested faster than mature
foliage of the same species. They calculated that digestion of the less digestible
foliage would yield energy so slowly that the sloth could starve to death following a meal of these leaves.

The emphasis on young foliage means that forest phenology could potentially have substantial effects on the distribution and abundance of arboreal folivores (Oates, 1977; C. Hladik, 1978; McKey, 1978; Oates et al., 1990). However, it has been postulated that limits to populations of arboreal folivores are determined by the availability and quality of "subsistence" foods such as mature foliage at times when young foliage or supplements such as fruits, flowers or seeds are not available (i.e. the time of lowest nutrient availability) (Cant, 1980; Cork and Pahl, 1984; Pahl, 1984; Terbough, 1986; Cork, 1992). Cork and Foley (1991) suggested that for this reason, comparison of diet quality between sites should concentrate on the mature foliage. This approach has been successful in explaining some patterns of colobid density (Oates et al., 1990).

In a number of situations food choice in arboreal folivores has been suggested to reflect the distribution and concentration of allelochemicals in the food items. However, in some of these cases there was little or no measurement of the distribution of the allelochemicals supposedly involved. Tannins generally act as anti-feedants in mammals (Mole and Waterman, 1987a; Bernays et al., 1989), though non-folivorous mammals display the strongest avoidance of tannins (Cork and Foley, 1991). McKey (1978) proposed that high phenolic levels in foliage, related to low soil resource availability (Section 2.2.1.2), forced colobids to select large quantities of seeds in their diet. This conclusion has been questioned by a number of authors who suggested that seeds are a common part of colobid diets and are consumed even in the presence of high quality foliage (Gautier-Hion, 1983; Oates et al., 1990). Oates et al. (1977, 1980) found that colobids selected a diet low in condensed tannins, though Oates (1977) also suggested that tannins may benefit Colobus guereza by reducing their propensity for "bloat" disorders which can be a problem in captive colobids (Oates, 1977; Clutton-Brock, 1977). Numerous studies have found little or no effect of phenolics on food selection by arboreal folivores (Hladik, 1977, 1978; Milton, 1979; Ganzhorn, 1988), although these results may be due to failure to assess the net balance of nutrient gain from food items (i.e. positive features of the tannin-rich foliage may have made it worth consuming despite the tannins, Janzen, 1978).

The role of alkaloids in food choice by arboreal folivores is unclear. Although Oates et al. (1980) found foliar alkaloid levels to be uncorrelated with food choice in Colobus (see also Hladik, 1977; Oates et al., 1977; McKey, 1978), other authors have suggested that diurnal variations in alkaloid concentrations (Robinson, 1979) may
explain the temporal patterns found in the feeding behaviour of folivorous primates (Wrangham, 1977; Clutton-Brock, 1977), and Ganzhorn (1988) suggested that dietary differences between lemur species were partly due to differential avoidance of alkaloids.

It is now generally accepted that primary nutrients and allelochemicals should not be considered separately, but as the balance of nutrient gain against the cost due to allelochemicals (Janzen, 1978). Thus several studies have refined the consideration of the link between leaf composition and food selection by folioves by combining positive (protein) and negative (fibre and tannins) aspects in a single foliage quality index, protein/(fibre + tannin) (McKey et al., 1981; Oates et al., 1990; Cork, 1992) or ratios of nutrients to allelochemicals (Cork, 1992; Hume and Esson, 1993). The correlation between site averages of protein/(fibre + tannin) and the biomass of folivorous colobid primates was very strong (Oates et al., 1990). The abundance of arboreal folivores in the Eucalyptus forests of eastern Australia is positively correlated with concentrations of nitrogen, phosphorus and potassium in foliage (Braithwaite et al., 1983; Cork, 1992), which are negatively correlated with the phenolic content of the foliage (Cork, 1992). Thus the ratios of nutrients to allelochemicals are high where marsupial folivores are abundant (Cork, 1992).

Arboreal folivores could also select foods to counteract specific dietary deficiencies, such as minerals or essential amino acid deficiencies (Oates, 1977; Hladik, 1978), or to alleviate the effects of allelochemicals (Janzen, 1978); some allelochemicals can act to counteract each other, such as tannin and saponin (Freeland et al., 1985b) or tannin and cyanogens (Goldstein and Spencer, 1985).

Arboreal folivores generally select foliage for high protein and digestibility and low fibre and other negative factors. These may best be expressed as a balance between the positive and negative aspects of each food item. Food selection in the koala is discussed more specifically in Chapter 8.

2.1.3.2. Hindgut vs foregut fermentation

In this Section I do not propose that hindgut fermentation is an adaptation to folivory, but that it provides certain advantages over a foregut fermentative strategy to small mammals faced with a highly fibrous diet.

Hindgut fermenting herbivores can be more efficient than foregut fermenters in utilizing easily digestible nutrients. In foregut fermenters, unless there is selective bypass of fermentation, the loss of energy from easily digestible and fermentable
foods can be 20% of that ingested (Demment and Van Soest, 1985). This energy loss is mainly due to production of carbon dioxide and methane during fermentation (Black, 1971; Demment and Van Soest, 1985).

Hindgut-fermenting herbivores, such as equids, have more rapid passage of digesta than foregut fermenters, such as ruminants, of similar body size (Equations 2.1 and 2.2, Duncan et al., 1990; Illius and Gordon, 1992). Consequently, they digest fibre to a lesser extent than do ruminants, but when faced with a high-fibre food source can maintain their intake at a higher level than foregut-fermenters, with lower retention time and the digestibility of fibre, and meet their requirements largely from the easily digested cell solubles (Duncan et al., 1990; Illius and Gordon, 1992). Very large herbivores (> 600 kg) are predominantly hindgut fermenters which are able to utilize abundant, high-fibre foods by using this strategy (Janis, 1976; Demment and Van Soest, 1985). Furthermore, given an abundant food source, hindgut fermenters of all sizes can extract more energy per day from food than can ruminants, although the differences are most pronounced for small body sizes (Duncan et al., 1990; Illius and Gordon, 1992). All arboreal foregut fermenters less than 15 kg in mass, except the sloth Bradypus which has very low energy requirements, are more or less dependent on some degree of frugivory or granivory to achieve high rates of fermentation (Cork, in prep). Many small (and some large) foregut fermenters also possess mechanisms to partially bypass the fermentation chamber, thus allowing high-quality food to escape fermentation and the related losses of energy. These include tragulids (Langer, 1974), macropodid marsupials (Hume, 1982, 1984, 1989; Hume and Carlisle, 1985; Freudenberger et al., 1989), and some ruminants (Hofmann, 1989). Little is known of the function of the colobid foregut, although Cork and Foley (1991) have suggested on the basis of anatomy (Langer et al., 1980; Langer, 1988) and diet (Langer, 1988; Hume, 1989) that it may be similar to that in the small macropods which have some sort of bypass mechanism (Hume et al., 1988). Ruminants have been thought to be excluded from the strategy of rapid digesta passage by the function of the reticulo-abomasal orifice which passes particles only when they attain some critical specific gravity. Before that point they must be broken down by rumination and fermentation and then passed through to the rest of the gut (Janis, 1976; Demment and Van Soest, 1985).

Cork and Foley (1991) attribute the prevalence of hindgut fermenters (except the sloth) among the most strictly folivorous mammals to the advantages of rapid throughput and enzymatic digestion before fermentation, and the allometric constraints on acquiring energy primarily from fermentation in small mammals.
Protection of fermenting micro-organisms from allelochemicals (Foley et al., 1987) may also favour hindgut fermentation among arboreal folivores.

The disadvantage of hindgut fermentation for utilizing fibrous diets is the inability of the herbivore to utilize proteins produced during microbial fermentation (Demment and Van Soest, 1985). Absorption of amino acids intact from the hindgut would effectively improve the quality of the dietary protein and remove dependence on dietary essential amino acids. There is no evidence for active uptake of amino acids in the hindgut of mammals (Foley and Cork, 1992; Hume et al., 1993), although recent studies of hindgut function in birds have shown that active transport of intact amino acids occurs to a substantial extent across the caecal wall (Moreto and Planas, 1989; Obst and Diamond, 1989). Instead of absorption of amino acids from the hindgut, some small caecum-fermenting mammals utilize bacterial protein by recycling caecal contents—caecotrophy (Homicke and Bjornhag, 1980; Chilcott and Hume, 1985).

2.1.3.3 Digesta retention and Caecotrophy


The need for a long retention time to maximize cell wall digestion (Section 2.1.2.3) conflicts with the strategy of rapid passage and reliance on cell contents (Section 2.1.3.2), rather than fermentation of fibre. Several marsupial folivores have been shown to combine the two strategies by selectively retaining fluid and fine particles of digesta. The koala, greater glider and ringtail possum all exhibit selective retention of fluid digesta markers (Cork and Warner, 1983; Chilcott and Hume, 1985; Foley and Hume, 1987a). In the koala the mean retention time of fluid digesta was 213 h while the mean retention of particulate digesta was measured as 99 h (Cork and Warner, 1983). The particulate marker used in their work, Ru-Phenanthroline, tends to transfer from particle to particle (Faichney and Griffiths, 1978), so transfer from the relatively few large particles to the many very fine particles in the gut may have biased the distribution of the marker towards fine particles. For this reason their measure of particle retention refers to fine particles (Cork and Warner, 1983). The selective retention mechanism operating in the koala, ringtail possum and greater glider seems to be similar to that in the rabbit (Bjornhag, 1972). A combination of antiperistaltic muscular contractions and a cycle of water between the colon and the caecum wash fine particles and soluble matter from the coarse particles back up into the caecum (Bjornhag, 1972).
The theoretical advantages of selective retention are substantial. First, large, fibrous and poorly digestible particles may be excreted relatively rapidly, thus reducing the gut-filling effect of the fibrous diet and allowing greater food intake (Cork and Warner, 1983; Chilcott and Hume, 1985; Foley and Hume, 1987a; Foley and Cork, 1992). Second, losses of valuable microbial protein and vitamins as well as endogenous proteins from the gut are reduced (Bjornhag, 1972). Koalas and greater gliders feeding on fibrous, tannin rich foliage have lower faecal nitrogen losses than brushtail possums (Cork, 1986; Foley and Hume, 1987b, c), presumably due to the effects of digesta separation, which is absent in the brushtail possum (Foley and Hume, 1987a).

In some arboreal folivores such as the ringtail possum, *Pseudocheirus peregrinus*, selective retention is combined with caecotrophy (Chilcott and Hume, 1985). Caecotrophes (Hornicke and Bjornhag, 1980), soft faeces derived largely from caecal contents, were produced by ringtail possums in their resting phase and ingested directly from the cloaca, while hard faeces were produced during the active phase and not consumed (Chilcott and Hume, 1985). The consumption of caecotrophes contributed considerably to both the energy and nitrogen budgets of the possums (Chilcott and Hume, 1984b, 1985), as is also the case in rabbits (Hornicke and Bjornhag, 1980). Chilcott and Hume (1985) attributed much of the ringtail possum's ability to survive on mature *Eucalyptus* foliage, despite its small size (approx. 0.7 kg), to the benefits of caecotrophy to their energy and nitrogen economy. Among the primate arboreal folivores, *Lepilemur* (similar in size to *Pseudocheirus*) is reported to practise coprophagy (Hladik *et al.*, 1971), although it is not known whether this is accompanied by selective retention and production of caecotrophes.

### 2.1.3.4 Other adaptations

Dentition is an important consideration in the adaptations of an animal to a particular diet. The rest of the alimentary tract must deal with the size classes of ingesta provided by the process of mastication. Kay and Hylander (1978) concluded that the optimal molar morphology for a folivorous diet emphasized shearing properties. They also drew attention to a trend of reduction in anterior dentition seen in *Lepilemur* and *Hapalemur*. Sanson (1980) also found that herbivores which were adapted to a high fibre diet used more of a cutting than a crushing action in mastication.

Small herbivores are constrained in their use of fibrous diets largely by their small gut capacity (Section 2.1.2.3). However, recent studies have forced a re-examination of the extent of limitations on gut capacity. In voles, *Microtus ochrogaster*, the size of
the caecum increased as food quality decreased, and the size of caecum and the small intestine increased with cold stress (Gross et al., 1985). Similar results have been shown in other mammals (Green and Millar, 1987; Hammond and Wunder, 1991; Loeb et al., 1991) and in birds (Moss, 1983). Toloza et al. (1991) showed that increases of this sort in small intestine size are accompanied by increased capabilities for nutrient absorption. Foley and Cork (1992) suggested that the plasticity in gut size and absorptive capacity found in recent studies may be sufficient to meet extra energetic demands such as those of reproduction or cold stress, but are not of great enough magnitude to offset all the effects of small size and poor diet on the ratio of metabolic rate to gut capacity.

2.1.3.5 Energetics

The other way in which arboreal folivores reduce their ratio of metabolic requirements to gut capacity is by reduction of metabolic requirements. Most arboreal folivores have been shown to have low basal metabolic rates (BMR) (McNab, 1978, 1980, 1986; Muller et al., 1983, 1985). McNab (1980, 1986) considered that low BMR of arboreal folivores was an adaptation to the allometric limitations of the folivorous diet, and suggested that BMR was negatively correlated with the proportion of leaves in the diet. These views have since been disputed by Elgar and Harvey (1987) and Harvey et al. (1991) who contended that there are no significant correlations of food type, independent of phylogeny and body mass, with BMR.

Nevertheless, BMRs reported for some arboreal folivores are extremely low (McNab, 1978). For instance, the BMR of Bradypus was only 42% of that predicted for a eutherian of that body mass (McNab, 1978) and body temperature was low and variable (30-37°C) (Montgomery and Sunquist, 1978). The tree kangaroo, Dendrolagus matschiei, and red panda, Ailurus fulgens, also have low BMR; 55 and 39% respectively of the Kleiber prediction (McNab, 1988). In addition, they are able to minimize heat loss at low temperatures, probably by reducing peripheral circulation while maintaining core temperature, leading to a reduction in metabolism instead of the expected rise (McNab, 1988). McNab (1988) predicted this ability to be widespread among tropical arboreal folivorous and frugivorous mammals, reflecting their adaptation to low energy expenditures. Dawson and Degabriele (1979) reported BMR in koalas to be only 74% of the level predicted for a marsupial of that mass (Dawson and Hulbert, 1970), and 53% of that predicted for a eutherian (Kleiber, 1975). These low rates were accompanied by low conductivity (McNab, 1978;
Degabriele and Dawson, 1979), and by a low but well-defended body temperature of about 36°C (Degabriele and Dawson, 1979; Nagy and Martin, 1985).

In contrast, several arboreal folivores have BMRs close to or slightly higher than that expected for mammals of their mass and taxonomy. The BMR of mantled howler monkeys, *Alouatta palliata*, was 5% above the Kleiber prediction of metabolic rate for eutherian mammals of their mass (Milton *et al.*, 1979). However, Muller *et al.* (1983) questioned Milton *et al.*'s (1979) results, suggesting that they may have been high due to the measurements having been taken in the animals' active period.

Among marsupials, the ringtail possum, *Pseudocheirus peregrinus*, and greater glider, *Petauroides volans*, have BMRs close to that expected for marsupials of their size (Kinnear and Shield, 1975; Foley, 1987 respectively). However, marsupials generally have a BMR about 70% of that in eutherians (Dawson and Hulbert, 1970), a comparatively low basal metabolism.

The energy requirements of free-existence, or field metabolic rate (FMR), of arboreal folivores are also low. It has been suggested that a low level of FMR is more important than a low BMR for survival on a diet poor in available energy, such as foliage, as basal metabolism does not include the energetic demands of thermoregulation, foraging, feeding and digestion (Hume *et al.*, 1984; Foley *et al.*, 1990). The three toed sloth, *Bradypus*, has an extremely low FMR; 21% of that predicted for other eutherians (Nagy and Montgomery, 1980). Field metabolism in *Alouatta palliata* is also low; 50-60% of the average eutherian level (Nagy and Milton, 1979a). The koala has a low FMR; 32 and 42% of the expected eutherian level (Nagy, 1987) for males and females respectively (Nagy and Martin, 1985).

Both the common ringtail possum and greater glider also have low FMRs compared with eutherians, but they are closer than that of the koala to the average for marsupials (Nagy, 1987; Munks, 1990; Foley *et al.*, 1990). While only the koala has a basal rate well below that expected for a marsupial, all marsupial folivores have an FMR which is a low multiple of BMR relative to other marsupials (Hume *et al.*, 1984; Munks, 1990; Foley *et al.*, 1990).

Low energetic expenditures have a number of advantages to an arboreal folivore and may be essential (Cork and Foley, 1991). Reduction of metabolic requirements reduces the ratio of requirements to digestive capacity, increasing the ability to meet their requirements from a poor diet. Reduction of required food intake due to reduced energetic requirements also reduces potential allelochemical intake and the associated detoxification (McNab, 1978). Low rates of basal metabolism are also associated with low rates of protein synthesis and low endogenous urinary nitrogen excretion.
(Smuts, 1935; Hume, 1982) leading to reduced maintenance nitrogen requirements. Cork and Foley (1991) considered BMR, digesta separation with or without caecotrophy, and effects of allelochemicals as the most important determinants of maintenance nitrogen requirements in arboreal folivores. Koalas have low endogenous urinary nitrogen excretion (Cork, 1986), although the detoxification and excretion of allelochemicals can raise urinary nitrogen excretion considerably (Foley and Hume, 1987b; Foley, 1992).

Activity is generally low in arboreal folivores. Both sloths and koalas are inactive for the majority of the daily cycle (Sunquist and Montgomery, 1973; Robbins and Russell, 1978; Lee and Martin, 1988; Mitchell, 1990b). Howler monkeys also show a low level of activity (Milton, 1979), while the degree of folivory is negatively correlated with time spent moving, daily movement and home range size in the primates generally (Clutton-Brock and Harvey, 1977). Muscle mass is also low in arboreal folivores (Grand, 1978), which would contribute to energy savings in basal metabolism and activity.

A number of arboreal folivores have been reported to behaviourally thermoregulate to some extent, presumably reducing the energetic requirements of physiological thermoregulation thereby. This includes sunbaking in colobids (Oates, 1977) and sloths (Montgomery and Sunquist, 1978).

Arboreal folivores also grow slowly (Russell, 1982a; Lee and Cockburn, 1985) and have slow rates of reproduction and population growth (McNab, 1978, 1980, 1986). McNab (1978, 1980, 1986) suggested that the low BMR of arboreal folivores is an adaptation to the energetic limitations of their niche, while slow growth and reproduction reflects the low BMR and the low availability of energy from a diet of foliage. Alternatively, the low metabolic rates found in marsupials may have preadapted the folivorous marsupials to their niche (Degabriele and Dawson, 1979). The low metabolic rates seen in arboreal folivores in general, and in koalas specifically, together with the difficulties of extracting energy from a foliage diet, have prompted several researchers to suggest that arboreal folivores survive on a very limited energy budget (McNab, 1978; Cork, 1981; Cork and Sanson, 1990).

In summary, arboreal folivores have low total energetic requirements (FMR), which have been achieved by the low values of the components of energy metabolism: BMR, activity, production and reproduction. Low BMR has the added advantage of reducing maintenance nitrogen requirements in arboreal folivores.
2.2 BIOLOGY OF THE KOALA

"...they have little, either in their character or nature to interest the naturalist or philosopher. As Nature however, provides nothing in vain, we may suppose that even these torpid senseless creatures are wisely intended to fill up one of the great links of animated nature."


2.2.1 Taxonomy

Classification (Strahan, 1983)

<table>
<thead>
<tr>
<th>Class</th>
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<tr>
<td>Subclass</td>
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<tr>
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<td>Diprotodonta</td>
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<tr>
<td>Family</td>
<td>Phascolarctidae</td>
</tr>
<tr>
<td>Genus</td>
<td>Phascolarctos</td>
</tr>
<tr>
<td>Species</td>
<td>Phascolarctos cinereus (Goldfuss)</td>
</tr>
<tr>
<td>Common name</td>
<td>Koala</td>
</tr>
</tbody>
</table>

The koala, *Phascolarctos cinereus*, is a diprotodont marsupial most closely related to the wombats among extant species (Strahan, 1978) on the basis of serology (Baverstock, 1984), sperm morphology (Harding and Aplin, 1990) and other morphological features (Archer, 1978; Aplin and Archer, 1987).

Archer *et al.* (1991) interpreted the low diversity and little changed morphology of phascolarctids over the last 25 million years as indicating that early koalas were probably very similar to extant koalas and may have specialised as *Eucalyptus* feeders early in their evolutionary history. Early forms of koalas (*Litokoala* sp) have been found among rainforest assemblages at the Riversleigh site, but their low abundance and diversity may indicate that they had specialised on the few eucalypts found in those rainforests and were able to take advantage of the spread of eucalypts as the continent dried out and sclerophyll forests spread (Archer *et al.*, 1991).

Subspecies of modern koalas have been described on the basis of size and pelage (Thomas, 1923 and Troughton, 1935 cited in Strahan, 1978), *P. cinereus adustus* in North Queensland and *P. cinereus victor* in Victoria, leaving *P. cinereus cinereus* as the nominate subspecies from N.S.W. However, these subspecies designations are not
in common use as they represent a continuous cline rather than separate groups (Strahan, 1978; Lee and Martin, 1988).

2.2.2 Koalas and Humans

Koalas were important to the Aboriginal inhabitants of eastern Australia as a food source as well as being spiritually significant. Aboriginal legends regarding the koala often relate it to water supplies or rain (Lee and Martin, 1988; Phillips, 1990). At the time of arrival of Europeans in Australia koalas were sparse and difficult to find. They were first mentioned in historical records in 1798, 10 years after first settlement (Iredale and Whitley, 1934), and John Gould found koalas to be very rare from 1838-1840 (Gould, 1863 cited in Warnecke, 1978). Parris (1948) attributed the early (1840s) rarity of koalas in the lower Goulburn district to hunting by Aboriginals. The fact that early sightings were in tall, dense forest where koalas would have been more difficult to find and catch tends to support this assertion (Warnecke, 1978). Later (1860s) abundance of koalas in the district was correlated with the decline of local Aboriginal populations (Parris, 1948).

In the late 1800s and early 1900s the trade in koala fur took a heavy toll on their population (Warnecke, 1978; Lee and Martin, 1988) and they became extinct in South Australia. They were protected by law in 1898 in Victoria, in 1903 in N.S.W. and in 1921 in Queensland, though one further one-month open season was declared in 1927 in Queensland (Phillips, 1990). In the late 1800s between 10 and 30 thousand koala skins were exported to Britain and in 1927 over 500,000 skins were taken in one month (Phillips, 1990). The decline in koala populations was also ascribed to diseases (Troughton, 1941) whose symptoms were similar to those of chlamydiosis (Obendorf, 1981).

Lee and Martin (1988) suggested that the major factors suppressing koala numbers after hunting ceased were destruction of suitable habitat and catastrophic fires. Disease, especially chlamydiosis, has been blamed for the ongoing decline of koala populations (Brown et al., 1984; Brown and Carrick, 1985), although the experience of researchers in Victoria (Lee and Martin, 1988) and Queensland (Gordon et al., 1990) suggest that disease does not pose a threat to the continued existence of wild koala populations.

In Victoria many koalas were translocated from the islands where they thrived to the mainland where local populations had declined (Warnecke, 1978). These introductions have been successful to the extent that, most of the remaining, secure and appropriate habitat in Victoria is populated by koalas (Martin, 1992). Koala
populations in secure island situations tend to boom and defoliate their food trees, resulting in population crashes (Martin, 1985a,b,c, 1992; Lee and Martin, 1988). There have thus been proposals to manage the Victorian populations by culling because now that appropriate relocation sites are scarce (Martin, 1992).

In N.S.W. koalas were perceived as common and secure "...at least by current standards of status..." (Gall, 1978) but are currently listed as "vulnerable and rare", largely on the basis of their large range reduction and scarcity in the south and west of the state (Reed et al., 1990; D. Lunney, pers. comm.). In Queensland, koalas have probably declined in the north of their range over the past 20 years but are widespread in the south-east of the state (Phillips, 1990). However, Phillips (1990) warned that the major centres of koala abundance in the north-east of N.S.W. and south-east of Queensland are threatened by urban development.

2.2.3 Ecology

2.2.3.1 Distribution and Abundance

Koalas have a patchy distribution throughout the eastern parts of the eastern states of Australia (Phillips, 1990) except Tasmania, where they are not found (Figure 2.1). Koalas are most abundant in the north-east corner of N.S.W., south-east corner of Queensland and at scattered locations throughout Victoria (see Gall, 1978; Gordon and McGreevy, 1978; Robinson, 1978; Warnecke, 1978; Reed et al., 1990, Phillips, 1990), and seem to be maintaining their broad distribution despite local reductions and reductions in the west and north of their range (Phillips, 1990). One of the most important findings of the 1986-7 survey was that extant koala populations were mainly to be found on privately owned rural lands (Reed et al., 1990).

The density of koalas in areas in which they occur varies both spatially and temporally from 0.4 to 8 koalas per hectare (Eberhard, 1978; Gall, 1980; Hindell, 1984; Martin, 1985c; Gordon et al., 1990; Mitchell and Martin, 1990; White and Kunst, 1990). Reported population fluctuations range from long-term decline (from 1.2 koalas per hectare to nearly zero over 11 years, Gordon et al., 1990) through fluctuations around a reasonably stable population (Eberhard, 1978; Gall, 1980; Mitchell and Martin, 1990; White and Kunst, 1990) to rapid population crashes (Martin, 1985c; Every, 1986; Gordon et al., 1988). Population crashes have been associated with defoliation of their favoured Eucalyptus species, either by drought (Gordon et al., 1988) or overbrowsing by koalas and subsequent starvation or dispersal (McNally, 1957; Martin, 1985a,b,c). Eberhard (1978) and Gall (1980) also
noted degrees of defoliation of favoured trees in their studies, but not to the extent of causing population crashes.

Figure 2.1 Distribution of koalas (from Lee and Martin, 1988). The black areas indicate the probable present distribution of koalas and the stippled areas indicate the range at the time of first European settlement.

The variation in density of koalas on both large and local scales suggests that there are different grades of koala habitat (Martin, 1985c; Mitchell, 1989; Gordon et al., 1988; Gordon et al., 1990). Mitchell (1989) observed dispersal of juvenile male koalas through habitat which he described as sub-optimal because there were no resident adult koalas and the density of preferred trees was very low. Mortality of koalas due to drought induced defoliation of preferred trees was lower around permanent water than in the surrounding areas, leading Gordon et al. (1988) to suggest that permanent water may be an important feature of primary habitat of koalas in southern-central Queensland. Martin (1985c) suggested that in Victoria the low coastal forests can support higher densities of koalas than can the taller inland forests.

2.2.3.2 Activity

Koalas are inactive for about 19-20 hours each day (15 of which they spend sleeping and 5 resting), feed for 4-5 hours and move for around 4-40 minutes (Robbins and Russell, 1978; Smith, 1979b; Nagy and Martin, 1985; Mitchell, 1990). For further information about behaviour of koalas see Smith (1979a,b,c, 1980a,b,c,) and Mitchell (1990a,b). The literature on home ranges of koalas is reviewed in Chapter 9.
2.2.3.3 Life history

The oestrus cycle of koalas (about 35 days) is the same length or longer than the gestation period (30-40 days) (V. Thompson, 1987; Handasyde et al., 1990). Lactation lasts about 360 days, first pouch exit is at around 210 days, and permanent pouch exit is at between 240 and 270 days (Russell, 1982a). Usually only one young is born, although there have been a number of reports of twins (McNally, 1957; R.W. Martin, pers. comm., I.D. Hume, pers. comm.). Pouch-young koalas consume solely milk until about 160 days, when they start to feed occasionally on a form of maternal faeces, "pap", and continue to do so until about the time of pouch exit (Fleay, 1937; Minchin, 1937; Smith, 1979c; Lee and Martin, 1988). Juvenile koalas start to eat some foliage after about 210 days (Smith, 1979c), so weaning is protracted, occupying one third of the lactation period.

Reproduction is prolonged in the koala, compared with either eutherians or marsupials of comparable size. While the gestation period is only 36 % of the predicted gestation for a eutherian of the same mass, 96 days (Equation 1.1, Millar, 1981), the time from conception to weaning, 395 days, is greater than that predicted for herbivorous eutherians, 242 days, or macropod marsupials, 315 days, of the same size (S. Thompson, 1987). This indicates that the duration of lactation in koalas is longer than in other mammals of similar size. The growth rate of koalas is also lower than eutherians or other marsupials of similar size (Russell, 1982a; Lee and Cockburn, 1985) and the relative mass of the young at weaning is low compared with most other marsupials except bandicoots (Russell, 1982a).

Female koalas may first breed toward the end of their second year, although females that breed early often lose that first young (Martin and Handasyde, 1990), and while males are probably sexually mature at two years (Handasyde et al., 1990; Martin and Handasyde, 1990), they probably have few opportunities to mate before 4-5 years (Martin and Handasyde, 1990). Females that lose young during the breeding season may return to oestrus and give birth again (Handasyde et al., 1990), although most young survive to pouch emergence (79-96 %) and independence (73-86 %, Martin and Handasyde, 1990). Females may produce one young per year until the age of 10 to 15 years, but fecundity declines with age over 10 years or with the presence of Chlamydia infection in the population. The longevity of females may be up to 18 years (Martin and Handasyde, 1990).
2.2.3.4 Feeding

Koalas usually consume foliage from 4 or 5 species of those Eucalyptus species present (Reed et al., 1990; ), and consume 50-60 species of Eucalyptus in N.S.W. (Reed et al., 1990) and over 30 in Victoria (Warnecke, 1978), but in the most recent national survey of koalas, 5 or 6 species of Eucalyptus in each state accounted for 60-90 % of all koala sittings (Phillips, 1990). The major tree species in which koalas were sighted were:

**South Australia**
- E. viminalis, E. leucoxylon, E. camaldulensis, E. ovata,

**Victoria**
- E. viminalis, E. obliqua, E. ovata, E. radiata, E. globulus,
- E. camaldulensis

**New South Wales**
- E. saligna, E. microcorys, E. punctata, E. pilularis,
- E. grandis, E. propinqua

**Queensland**
- E. teretecornis, E. crebra, E. populnea, E. camaldulensis,
- E. maculata, and E. propinqua.

However, as koalas use different species of trees in each area and the above list may be biased by the distribution of observers, the preferences of koalas for species of Eucalyptus should be examined on a local rather than national scale.

The factors influencing foliage preferences of koalas are discussed in Chapter 8.

2.2.3.5 Digestion and Energetics

The koala is a hindgut fermenter, with extensive enlargement of the caecum. Mackenzie (1918) suggested that the caecum of the koala is proportionally the largest in any mammal. Fermentation of fibre occurs in both the caecum and proximal colon (Cork and Hume, 1983). However, koalas obtain their energy largely from the cell solubles of Eucalyptus foliage- the non-structural carbohydrates, lipids and protein (Cork et al., 1983). Microbial production of volatile fatty acids provides less than 10 % of their digestible energy (Cork et al., 1983; Cork and Hume, 1983).

Digesta passage is very slow in the koala. The mean retention time (average time of residence of digesta) of a fluid marker (213 hours) was greater than that of particles (100 hours) in captive koalas, indicating selective retention of fluid digesta in the caecum and proximal colon (Cork and Warner, 1983). Selective retention of fluid digesta has also been found in the greater glider (Foley and Hume, 1987a) and ringtail possum (Chilcott and Hume, 1985), both marsupial folivores. Selective retention may function to minimize losses of microbial protein, as well as reducing the gut-filling effect of fibre (Cork and Warner, 1983; Section 2.1.3.3; Chapter 7). Due to the high degree of lignification of fibre in Eucalyptus foliage, the time of residence of fibre in
the fermentation chamber of the koala's gut is greater than that required for maximal
digestion of the fibre (Cork and Sanson, 1990). Therefore the long retention time and
selective retention may function more to reduce faecal nitrogen losses of microbial
protein and allow de-tanning of protein complexed with dietary tannins (O'Brien et
al., 1986) than to allow maximal digestion of fibre.

The dentition of koalas seems well suited to their strategy of utilizing mainly cell
contents. The shearing action of mastication in the koala ruptures the cells and
reduces the cell walls to small fragments (Lanyon and Sanson, 1986a, Cork and
Sanson, 1990), 60 % of which are in the size range selectively retained (Lanyon and
Sanson, 1986b). The pattern of wear initially increases the length of cutting edges on
the molars and then decreases them, but serves to maintain the efficiency of
mastication for most of the koala's life (Lanyon and Sanson, 1986b).

The BMR of koalas (151 kJ.kg$^{-0.75}$.d$^{-1}$) is only 74 % of the average for marsupials of
the same mass (Degabriele and Dawson, 1979) and the FMR (330-440 kJ.kg$^{-0.75}$.d$^{-1}$)
is also low (Nagy and Martin, 1985, see Chapter 6). Because of the low rate of
metabolism urinary losses of endogenous nitrogen are low (Cork, 1986). This keeps
the maintenance nitrogen requirements (271 mg N. kg$^{-0.75}$.d$^{-1}$) relatively low despite
high faecal nitrogen losses due to the high fibre and tannin content of Eucalyptus
foliage (Cork, 1986). About 80 % of the urea produced by the deamination of protein is recycled to the hindgut, providing a nitrogen source for the fermenting bacteria and possibly raising the rate of fermentation, thereby aiding both the koala's nitrogen and energy economies (Cork, 1981).

The phenolics and essential oils found in Eucalyptus foliage affect digestion and
metabolism in the koala. Essential oils in Eucalyptus foliage increase urinary
nitrogen losses in ringtail possums and greater gliders and may challenge their acid-
base homeostasis (Foley, 1987; Foley, 1992). Tannins may elevate faecal nitrogen
losses (Cork, 1986; Foley and Hume, 1987c). Detoxification and excretion of
essential oils and hydrolysable phenolics also have energy and nutrient costs
(Eberhard et al., 1975; Baudinette et al., 1980; Cork et al., 1983; Foley, 1987, Dash,
1988; Foley, 1992), although Cork and Sanson (1990) suggested that they are minor
compared with the reduction in metabolizable energy content of Eucalyptus foliage
and nitrogen losses due to allelochemicals (Section 2.1.2.2).
2.3 SYNTHESIS: REPRODUCTION ON A LIMITED BUDGET

Reproduction, especially at the peak of lactation, places large energetic demands on female mammals. Increases in energy requirements during the peak of lactation range from 60 to 200% (Gittleman and Thompson, 1988). In most mammals the energetic requirements of lactation are met primarily by an increase in dietary energy intake, so that allocation of energy to reproduction in small herbivores may be limited primarily by limits to dietary energy intake (Kenagy et al., 1990).

The koala is a marsupial arboreal folivore subject to stringent allometric constraints on its acquisition of energy from its diet of *Eucalyptus* foliage, which is poor in available nutrients. The koala is able to survive on such a diet despite allometric constraints largely due to its energetically conservative characteristics and its digestive adaptations. That is, it minimizes energetic expenditure while maximizing energy extraction, but still is likely to exist on a fine energetic balance (Cork and Sanson, 1990). The magnitude of allocation of energy to reproduction in koalas and the sources of expenditure are likely to reflect constraints on their ability to extract nutrients from their diet.

How is an arboreal folivore like the koala able to meet the energy requirements of reproduction, especially at peak lactation? Other aspects of koala energetics suggest that they are "energetic minimalists"; they minimize daily energy expenditure and lactation is prolonged, so it is likely that allocation of energy to reproduction may be of low magnitude and spread over a long period, thus minimizing daily requirements for lactation. Chapters 4 and 5 of this thesis examine the magnitude and temporal pattern of allocation of energy to lactation by koalas, while Chapter 6 concerns the sources of energy for lactation. Later chapters are concerned with the effects of lactational requirements on aspects of the physiology and ecology of koalas; digesta passage (Chapter 7), diet composition (Chapter 8) and ranging behaviour (Chapter 9).
CHAPTER THREE

GENERAL MATERIALS AND METHODS.

3.1 INTRODUCTION

This section refers only to those materials and methods used throughout the study which form a basis for the specialised techniques used in the different aspects of the investigation. Those techniques are detailed in the appropriate chapters.

3.2 THE STUDY SITE

3.2.1 Location

Fieldwork for this study was carried out between August 1989 and February 1992, on private property near the town of Nowendoc on the south-eastern corner of N.S.W.'s New England Tableland. The site comprised remnant patches of woodland among improved pastures used for the production of beef cattle, at an altitude of 900-1000 m (Figure 3.1).

Figure 3.1 Looking south across the study site. The grid was among the trees in the midground.
Preliminary observations of the distribution of koalas in the area were made in May and August 1989, before a grid was set out as a basis for subsequent locational work. The grid was oriented to take advantage of natural and existing features relating it to aerial photographs and the topographic map, and to allow for the irregular and patchy nature of the remnant koala habitat.

The area was marked out with a grid of one hectare squares (100 by 100m). Grid points were marked using 2 m steel posts marked with flagging tape and survey paint, and labelled with aluminium tags using a six digit X-Y coordinate system (Figure 3.2). Most preliminary observations were made on the valley floor, and the position of the grid reflects the distribution of koalas over the larger area.

![Figure 3.2 The study area, showing the grid in relation to the patches of trees.](image-url)
3.2.2 Climate

Climatic data (Table 3.1) were taken from records at a Forestry camp approximately six km to the east of the site, at 1010 m elevation.

Table 3.1 Temperature and precipitation at Riamukka camp.¹

<table>
<thead>
<tr>
<th>Month</th>
<th>Rainfall (mm)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Max.</td>
</tr>
<tr>
<td>January</td>
<td>174</td>
<td>25</td>
</tr>
<tr>
<td>February</td>
<td>179</td>
<td>24</td>
</tr>
<tr>
<td>March</td>
<td>144</td>
<td>22</td>
</tr>
<tr>
<td>April</td>
<td>77</td>
<td>19</td>
</tr>
<tr>
<td>May</td>
<td>109</td>
<td>16</td>
</tr>
<tr>
<td>June</td>
<td>78</td>
<td>12</td>
</tr>
<tr>
<td>July</td>
<td>62</td>
<td>13</td>
</tr>
<tr>
<td>August</td>
<td>37</td>
<td>16</td>
</tr>
<tr>
<td>September</td>
<td>60</td>
<td>19</td>
</tr>
<tr>
<td>October</td>
<td>114</td>
<td>21</td>
</tr>
<tr>
<td>November</td>
<td>113</td>
<td>25</td>
</tr>
</tbody>
</table>

¹ Mean monthly values from 10 years of records (Forestry Commission, 1987)

The site is in a summer rainfall area (Table 3.1), and is relatively dry in winter and early spring. The pattern of rainfall has the potential to affect the quality, quantity and timing of foliage available to koalas on the site (Chapter 8).

3.2.3 Vegetation

Within the study area, koalas were primarily found in the patches of *Eucalyptus* woodland, canopy height 10-20 m, that was found in the valley. Four main species of *Eucalyptus* were present, with a fifth represented by scattered individuals, and few non-eucalypts (Table 3.2).
Table 3.2 Predominant Available Tree Species

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eucalyptus pauciflora</em></td>
<td>Snow Gum</td>
<td>50</td>
</tr>
<tr>
<td><em>Eucalyptus acaciiformis</em></td>
<td>Wattle-leafed Peppermint</td>
<td>17</td>
</tr>
<tr>
<td><em>Eucalyptus radiata</em></td>
<td>Narrow leafed Peppermint</td>
<td>16</td>
</tr>
<tr>
<td><em>Eucalyptus stellulata</em></td>
<td>Black Sallee</td>
<td>6.3</td>
</tr>
<tr>
<td><em>Eucalyptus viminalis</em></td>
<td>Manna or Ribbon Gum</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Allocasuarina littoralis</em></td>
<td></td>
<td>6.3</td>
</tr>
<tr>
<td><em>Acacia falciformis</em></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td><em>Acacia melanoxylon</em></td>
<td></td>
<td>1.8</td>
</tr>
<tr>
<td><em>Banksia integrifolia</em></td>
<td></td>
<td>1.6</td>
</tr>
</tbody>
</table>

1 Classification according to Williams (1985).
2 Proportions based on occurrence within the study grid (see Chapter 8).

On the slopes and ridges overlooking the valley a taller stringybark *Eucalyptus* association dominated including:

*Eucalyptus cameronii*
*Eucalyptus andrewsii*
*Eucalyptus obliqua*
*Eucalyptus laevopinea*
*Eucalyptus caliginosa*
*Eucalyptus radiata*
*Eucalyptus saligna* and
*Acacia falciformis*

However, little work was carried out in these areas as koalas were uncommonly sighted there and no attempt was made to exhaustively catalogue the species present.

### 3.3 RADIO-LOCATION AND MAPPING

The koalas used in the intensive studies of movement, metabolism, milk production and passage rates were each fitted with a collar mounted radio-transmitter for radio-location. The transmitters used were two stage with a whip antenna, produced by Titley Electronics, Ballina NSW.

The advantage of this model was that it was built into a screw topped aluminium casing, allowing battery changes to be made quickly and easily in the field, if
necessary. The collar was made from "flexible PVC" (kindly donated by MTI Qualos Pty Ltd) lined with sheepskin, the wool side to the koala. They were constructed in advance and sized to each individual before fastening at the rear with an aluminium pop rivet. The total weight as fitted was about 50g.

The collars were fitted loosely to avoid chafing and could be slipped on and off over the koala's head for inspection. The transmitter was fixed to the front of the collar and rested under the koala's chin with the antenna threaded through the collar to the back and protruding behind the koala's head for about 50 mm. Battery life was 8-9 months, but they were replaced on a regular basis at 7 months, and range was 3-4 km under good conditions.

All locations were confirmed visually. The co-ordinates of trees used by koalas were determined by taking compass bearings to two of the marked grid-points, or measuring both the distance and the compass bearing to one grid-point. Both Telonics and AVM brand radio-receivers were used to locate koalas.

3.4 CAPTURE AND HANDLING OF KOALAS

3.4.1 Capture

After location of a koala, the tree it occupied was measured, mapped, labelled, and observations were made of the climatic conditions, and the behaviour and position of the koala. When necessary, koalas were captured by the method of Martin and Handasyde (pers. comm., 1989). In this method the catcher climbed the tree to within at least 7m of the koala. Ropes, harness, and rock-climbing and abseiling techniques were used to ensure the safety of both the climber and koala during the procedure.

When within range of the koala an extendable aluminium pole (manufactured by P. Domelow, Monash University) was used to position a brightly coloured flag above or beyond the koala. Koalas backed away from this kind of disturbance, toward the catcher. At the appropriate juncture, when the koala was between 2 and 5 metres away, the flag was replaced with a noose of soft rope with a stop-knot to prevent complete closure. This was manoeuvred over the koala's head, the pole withdrawn and tension on the rope used to prevent the koala moving away. Using the flag again the koala was worked all the way back to the climber, coaxed into a hessian bag and lowered to a waiting assistant.
The procedure took between ten minutes and an hour once the climber was in position. The possible effects of stress on the animals were of some concern, and so a record was kept of the "capture impact" in each instance. This was a four category subjective index of the stress of capture based on the time taken and the reaction of the animal (Table 3.3).

Table 3.3 The capture impact index.

<table>
<thead>
<tr>
<th>Capture Impact</th>
<th>Time (min.)</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 10</td>
<td>No crying or urinating</td>
</tr>
<tr>
<td>2</td>
<td>10-30</td>
<td>Koala may urinate.</td>
</tr>
<tr>
<td>3</td>
<td>30-60</td>
<td>Koala may cry or urinate.</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 60</td>
<td>Koala may cry or urinate, usually moves freely around tree canopy</td>
</tr>
</tbody>
</table>

Most captures took about 20 minutes (Capture impact=2) and were apparently relatively unstressful. In no case was there any deleterious long term effect on a koala that could be attributed to the stress of one or repeated captures.

3.4.2 Handling of captured koalas

Once on the ground the koala was weighed and sedated with valium-Diazepam 0.5 mg.kg\(^{-1}\) body weight (T. Bellamy, pers. comm.) injected intramuscularly to the sartorius muscle on the top of the thigh. Once the sedative had acted (approx. 2-3 min.), measurements were taken and its health assessed. Measurements included head length (occiput to tip of nose) and width (over the zygomatic bones), body length from the base of the tail to the occiput, nipple length and mammary diameter in females, and scrotal length and width and the length and width of the sternal gland in males. The health assessment included heart and respiration rates, deep rectal temperature, tooth wear, external condition and body condition. Body condition was assessed on a subjective 4 point scale based on the mass of the *muscularis trapezius* over the scapula (Wood, 1978). Koalas were aged by the scheme of Martin (1981), based on the sequential wear of the upper left premolar and molars. This technique was tested with known age animals at Featherdale Wildlife Park in western Sydney and found to be relatively accurate and consistent, although the accuracy declined with increasing wear and age.

At their first capture koalas were ear tagged with coloured plastic ear tags (Daken sheep tags, Figure 3.3). Tags were applied to a colour coding scheme that allowed
individuals to be identified at a distance (ie. from the base of the tree). All males were tagged with combinations of only yellow, blue and pink in the left ear, while all females were tagged with combinations of only red and green in the left ear, while the right ear in both sexes was tagged with any combination of the four colours. Tags were also numbered sequentially so that any koala could be identified from a single tag on close inspection. Juveniles were initially marked with small numbered aluminium tags until about 11 months, when they could be permanently marked.

![A marked male koala, showing the eartags and radiotransmitter.](image)

**3.5 EXPERIMENTAL DESIGN AND TIMING**

Water turnover, milk production and daily energy expenditure were measured on 13 occasions during 1990, 1991 and 1992 in radio-collared female and juvenile koalas resident on the study site (Table 3.4).
Table 3.4 The times of measurement of isotope fluxes in free-living koalas at Nowendoc.

<table>
<thead>
<tr>
<th>Year</th>
<th>Season</th>
<th>Month</th>
<th>Water Flux</th>
<th>FMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>Winter</td>
<td>June</td>
<td>F, J</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>July</td>
<td>F, J</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Winter/Spring</td>
<td>August/September</td>
<td>F, J</td>
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<tr>
<td></td>
<td>Spring</td>
<td>October</td>
<td>F, J</td>
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<tr>
<td></td>
<td>Spring</td>
<td>November</td>
<td>F, J</td>
<td>F</td>
</tr>
<tr>
<td>1991</td>
<td>Summer</td>
<td>January</td>
<td>F, J</td>
<td>F</td>
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<tr>
<td></td>
<td>Autumn</td>
<td>May</td>
<td>F, J</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>July</td>
<td>F, J</td>
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<td></td>
<td>Winter/Spring</td>
<td>August/September</td>
<td>F, J</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>Sept./Oct.</td>
<td>F, J</td>
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</tr>
<tr>
<td></td>
<td>Spring</td>
<td>Oct./Nov.</td>
<td>F, J</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>December</td>
<td>F, J</td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td>Summer</td>
<td>January</td>
<td>F, J</td>
<td>F, J</td>
</tr>
</tbody>
</table>

F = Measurements made on adult females.
J = Measurements made on juvenile females and males.
FMR is Field metabolic rate.

Estimation of water flux and field metabolic rate were made in all adult females, lactating and non-lactating, known to be present on the study area at the times in Table 3.4.

The times of FMR estimation were planned to coincide with the time of peak lactation predicted from the observed developmental stages of juveniles on the site and the published time course of lactational output in Tammar Wallabies (Cork and Dove, 1989) (August/September), the predicted time of least lactational demand (January), as well as several points before and after peak output (May, June, July, November and December). In 1991, FMR of adult females could not be measured concurrently with that of suckling juveniles due to the problem of isotope transfer in the milk.
CHAPTER FOUR

MILK COMPOSITION IN THE KOALA

4.1 INTRODUCTION: FACTORS AFFECTING MILK COMPOSITION

Lactation in domestic mammals is an important process in agricultural production and aspects of milk composition, physiology and biochemistry of milk secretion, and energetics and nutrition of dairy animals have received considerable attention from researchers (Jenness and Patton, 1959; Kon and Cowie, 1961; Larson and Smith, 1974; Garnsworthy, 1988). However, beyond the scope of agricultural and domestic species there has been little detailed research of milk composition (Oftedal, 1984).

The composition of marsupial milk has been examined in a number of macropodoids (Poole et al., 1982; Green, 1984; Green and Merchant, 1988; Crowley et al., 1988; Smolenski and Rose, 1988; Merchant, 1989; Merchant et al., 1989; ), most notably *Macropus eugenii* (Messer and Green, 1979; Green et al., 1980; Cork and Dove, 1989), two didelphids (Bergmann and Housley, 1968; Green et al., 1991b), dasyurids (Green, 1984; Green et al., 1987), the northern brown bandicoot, *Isoodon macrourus* (Merchant and Libke, 1988; Merchant, 1990), the numbat, *Myrmecobius fasciatus* (Griffiths et al., 1988), the sugar glider, *Petaurus breviceps* and the common wombat, *Vombatus ursinus* (Green, 1984). Milk composition has also been examined in three species of marsupial arboreal folivores, the koala (Green, 1984; Marshall et al., 1990), the common ringtail possum, *Pseudocheirus peregrinus* (Munks et al., 1991) and the common brushtail possum (Cowan, 1989; Crisp et al., 1989a).

Within eutherian phylogenetic groups milk composition is relatively constant, but it differs widely between groups (Oftedal, 1984). Differences between and within phylogenetic groups are correlated with physiological factors and life history. Physiological factors affecting milk composition include neonatal mass (Blaxter, 1961; Payne and Wheeler, 1968) and suckling frequency (Ben Shaul, 1962; Oftedal, 1984). Blaxter (1961) suggested that small neonates with high surface area and high metabolic rate would require energy-dense milk, a pattern which was empirically confirmed (Payne and Wheeler, 1968). Ben Shaul (1962) suggested that, due to gut size limitations of the young, the milk of species that suckle infrequently should be high in energy to allow the young to rapidly ingest its requirements.

It has been proposed that milk concentration (Brody, 1945; Baverstock et al., 1976), and specifically the proportion of protein in milk solids (Bernhart, 1961; Bjornhag et
al., 1979), is correlated positively with the growth rate of young, though Oftedal (1981, cited in Oftedal, 1984) questioned this conclusion, suggesting that it was merely a consequence of the relationships of both milk composition and relative growth rate to size.

Water conservation and thermoregulatory requirements of the young are also correlated with aspects of milk composition. High solids and fat content of pinniped milk may be due to the need for maternal water conservation (Le Boeuf et al., 1972; Oftedal et al., 1987) or the need for rapid growth and adipose deposition for insulation in the young (Jenness and Sloan, 1970; Schmidt-Neilsen, 1979). Baverstock et al. (1976) found that several desert-adapted rodents increased milk solids content in response to water restriction, though desert rodents in general do not produce milk more highly concentrated than other similar sized rodents (Jenness and Sloan, 1970; Baverstock et al., 1976; Oftedal, 1984). Water restriction causes the production of more dilute milk in camels (Yagil and Etzion, 1980), humans and cattle (Yagil et al., 1986), and possibly harp seals (Lavigne et al., 1982). This may be due to the partial cross-reactivity of anti-diuretic hormone (ADH) and oxytocin (Guyton, 1986), meaning that prolactin and oxytocin could act similarly to aldosterone and ADH, taking up water from the intestine and kidney for secretion in milk (Yagil et al., 1986). The increase in body water observed during lactation in some desert-adapted ungulates may act as a reservoir for milk water (Maltz and Shkolnik, 1984).

Composition of the diet can affect composition of milk within a species (see Thomas and Martin, 1988 for a review on dairy species). The best information on the influence of diet on milk composition comes from domestic ruminant dairy species, but this information can be difficult to interpret due to the modifying effects of ruminal fermentation. Thomas and Chamberlain (1984) reviewed studies of dairy cattle in which nutrient supply to the small intestine was manipulated by infusion directly to the abomasum, thus avoiding alteration in the rumen, and concluded that concentration and yield of milk fat and protein change in response to specific nutrients. The causes of changes in milk composition are complex and dependent on the nature of the normal diet as well as the added nutrient (Thomas and Chamberlain, 1984).

Marsupial milk undergoes profound changes in composition over the course of lactation (Green, 1984; Green and Merchant, 1988). It is often inferred that the changing composition of marsupial milk is closely related to the changing requirements of the young (Tyndale-Biscoe and Janssens, 1988; Green and Merchant, 1988), which is nourished by milk for most of its development (Russell, 1982a).
However, the phenomenon of asynchronous concurrent lactation (Lincoln and Renfree, 1981) and results of the experimental transfer of pouch young (Merchant and Sharman, 1966; Findlay, 1982) suggest that the composition and yield of milk from a marsupial mammary gland are intrinsic properties of that gland and its stage of development.

The pattern of changes in milk composition is similar in most marsupial species which have been studied, despite variation in ecology and life history (Green, 1984; Green and Merchant, 1988; Figure 4.1). There are, however, some differences between species. Among macropods, Merchant (1990) and (Merchant et al., 1989) suggested that maximum solids concentrations may be inversely correlated with body size, and Green and Merchant (1988) noted an inverse correlation of peak solids and duration of lactation.

Figure 4.1 Proximate composition of milk of four marsupials. a) Isoodon macrourus, b) Dasyurus viverrinus, c) Trichosurus vulpecula, d) Macropus eugenii. S solids, L lipids, P protein, C carbohydrate (taken from Green and Merchant, 1988).
Milk composition in three marsupial folivores appears to conform to the general marsupial pattern (Green, 1984; Cowan, 1989; Marshall et al., 1990; Munks et al., 1991).

Munks et al. (1991) suggested that differences in the milk composition of wild and captive Pseudocheirus peregrinus may be related to differences in diet composition. Green and Merchant (1988) speculated that the unusual fatty acid composition of milk from Trichosurus and Phascolarctos compared with other marsupials, may be due to their folivorous diet. The fatty acid composition of dietary fat has been shown to affect fatty acid composition in the two monotremes and the numbat (Griffiths et al., 1984, 1988; Gibson et al., 1988)

Components of milk may be important in the preparation of juvenile folivores for the allelochemicals in their adult diet. MacLennan et al. (1983) found phenolic compounds and terpenes in the milk of brushtail possums. They suggested that allelochemicals passed from mother to young in the milk may function to induce the detoxification enzymes of the young, preparing them for intake of foliage.

The dietary niche of koalas and related energetic constraints make the composition of their milk an important part of their allocation of energy to reproduction. However there is little information on changes in koala milk composition during lactation. This chapter reports on the milk composition of free-living koalas. Measurements were carried out concurrently with measurement of their energy requirements (Chapter 6) and milk production (Chapter 5).

4.2 MATERIALS AND METHODS

4.2.1 Sampling

Milk samples were collected from koalas during middle and late lactation in 1990 and 1991, at the times of capture for determination of isotope turnover (Table 3.4). Pouch young koalas were not detached from the teat for the purposes of milk sampling, so the samples come only from lactational phases 2b and 3 (Tyndale-Biscoe and Janssens, 1988).

Sampling methods can influence the composition of the milk obtained. Oftedal (1984) highlighted methodological problems with the available literature on milk composition of wild mammals. Milk composition varies with the stage of lactation, time since the last suckling and the size of sample (Oftedal, 1984). The sampling procedure was designed to minimize these problems.
Milk was sampled just before the equilibration blood sample (see Section 6.2.1.2) for FMR or water intake measurements. This meant that there was at least 2h since the young last suckled.

The female koala was given an intramuscular dose of Diazepam (Valium, 0.5 mg.kg\(^{-1}\)) immediately before sampling the milk. Sampling without using the sedative was stressful, difficult and impractical. In the case of females previously sedated (for the measurement of isotope fluxes, Chapters 5 and 6), the level of sedation was assessed from their behaviour, and if necessary they were given a second dose of the sedative.

The lactating females were then given an intramuscular dose of oxytocin (Syntocin, Ilium, 1 i.u./kg). Milk began to flow 30-60 seconds later. During this time the teat was cleaned with an alcohol swab and dried. Milk was expressed by repeatedly squeezing from the base of the teat toward the apex, and collected in 1 ml or 5 ml plastic containers (Figure 4.2). This procedure was continued until little or no more milk could be expressed, thus avoiding the errors associated with incomplete sampling of the milk (Oftedal, 1984). High doses of Oxytocin can affect the composition of milk (Oftedal, 1984); however, without the hormone little or no milk could be expressed.
Milk samples were placed on ice until storage at -20°C at the end of the day.

4.2.2 Chemical Analysis

Koala milk is viscous and readily separates to aqueous and lipid phases. This presented considerable difficulties to the simple task of homogenizing and sampling. To minimize phase separation the samples were quickly brought to 37°C in a water bath, homogenised and immediately subsampled. Because of the high viscosity of koala milk, sampling with conventional negative-pressure auto-pipettes led to highly variable results. Therefore, positive displacement capillary pipettes (American Dade) were used to sample milk for all analyses. When more than one sample container was necessary during collection, the entire sample was mixed before subsampling. All analyses were performed in duplicate.

4.2.2.1 Milk Solids

Milk solids content was determined gravimetrically by weighing a 50 or 100 μl aliquot of milk, drying at 100°C for 24 h in a pre-dried glass tube, and reweighing after cooling in a dessicator.

4.2.2.2 Milk Lipid

The lipid content of milk samples was determined gravimetrically by extracting milk solids with diethyl ether (Green et al., 1991b). Diethyl ether (1.5 ml) was added to the tubes containing milk solids (from the determination of milk solids, Section 4.2.2.1), and the tube was agitated for 30 min before decanting the ether. The ether extraction was repeated a further three times before drying the residue for 24 h at 100°C, cooling in a dessicator and weighing.

4.2.2.3 Milk Carbohydrates

The carbohydrate content of koala milk was determined by the Phenol-sulphuric acid method as modified by Messer and Green (1979).

Koala milk (5 μl) was diluted with water (2 ml) for this analysis. Phenol solution (1 ml, 3.55 %) was added to 200 μl diluted milk in a test tube. Sulphuric acid (3 ml, 92 %) was added to the tube rapidly and mixed. The contents of the tube were allowed to cool at room temperature for 30 min before measuring absorbance at 490 nm (A₄₉₀) in glass cuvettes with a Varian DMS-100 UV-Vis Spectrophotometer. A standard curve was constructed by adding 40, 80, 120, 160, and 200 μl of a Lactose monohydrate standard solution (0.5 mg.ml⁻¹) to separate test-tubes, making them up
to 200 µl with distilled water, and then treating them as for the samples. A concentration vs absorbance equation was determined from the standards, using linear least squares regression; this equation was used to determine the concentration of carbohydrate in the samples as lactose monohydrate equivalents. A separate standard curve was constructed for each batch of samples.

The carbohydrate concentration of the milk sample was measured as percentage weight per volume. This was corrected to the percentage weight for weight using the previously measured specific gravity (Section 4.2.2.1).

Thin-layer chromatography was used to determine the qualitative composition of the milk carbohydrates by the method of Crisp et al. (1989c). Milk was diluted with water (1:10), and 2 µl aliquots spotted onto precoated silica gel chromatography plates (Merck 5553), then dried with a hair dryer. Standards (1 µl) were spotted at each end of the line of sample spots. The standards were a mixture of mono- and disaccharides, and oligosaccharides isolated from the milk of tammar wallabies (Messer et al., 1982). The composition of standard one was N-acetyl glucosamine (0.4 %), glucose (0.2 %), galactose (0.2 %), lactose (0.2 %), 3'-galactosyllactose (0.3 %), 3',3''-digalactosyllactose (0.4 %) and 3',3'',3'''-trigalactosyllactose (0.4 %). Standard two consisted of 3'-galactosyllactose (0.2 %), 3'-di to penta galactosyllactose (all 0.2 %). The solvent used was propan-2-ol: acetone: 0.1 M lactic acid, 4:4:2 (v/v) and the carbohydrates were detected by spraying with aniline diphenylamine reagent (Hansen, 1975).

### 4.2.2.4 Milk Protein

Protein was measured in koala milk by the Coomassie Blue protein-dye binding method (Bradford, 1976). The protein reagent was prepared by dissolving Coomassie Brilliant Blue G250 (100 mg) in ethanol (50 ml, 95 %), adding phosphoric acid (100 ml, 85 %), diluting to 1000 ml with distilled water and mixing well. This solution was then filtered and stored in the dark.

For protein analysis, as for carbohydrate analysis, the milk was diluted (5 µl in 2000 µl water) and 50 µl aliquots pipetted into test tubes. The volume was made up to 200 µl with distilled water, 3 ml of protein reagent added and the contents vortexed. Because the colour in the reaction faded over time, samples were analysed in groups of seven, each with a standard curve, so that the absorbance was measured at 595 nm (A595) 5-20 min after addition of the protein reagent. The standard was Bovine
Serum Albumin (BSA) (0.10 mg.ml\(^{-1}\)), prepared by dissolving BSA (25 mg) in water (25 ml), then diluting 1:10. The standard curve was prepared by adding 40, 80, 120, 160, and 200 µl of standard to different test-tubes and making up to 200 µl with water before addition of the protein reagent.

The standard curve was determined by fitting a least squares regression line and protein concentration of the samples estimated using that relationship. A separate standard curve was constructed for each batch of samples.

The protein concentration obtained was converted to units of % w/w (i.e. g/100g) using the previously determined value of specific gravity (Section 4.2.2.1).

4.2.2.5 Energy

The energy content was calculated from the measured milk composition using energetic equivalents of 38.1 kJ.g\(^{-1}\), 24.6 kJ.g\(^{-1}\), and 16.5 kJ.g\(^{-1}\) for lipid, protein and carbohydrate respectively (Perrin, 1958).

4.2.3 Ageing the young.

Pouch and back-young koalas can be aged from the relationship of head-length to age determined from known age animals (Martin, 1983b; Martin and Handasyde, 1990). However, application of Martin and Handasyde's (1990) equation to those young for which date of birth was known within 7 days resulted in underestimation of their age. Therefore a relationship similar to that of Martin and Handasyde (1990) was derived for the young of the study population using data from eight animals of known age (Equation 4.1):

\[
\text{Age (days)} = 0.023 \times (HL)^2 + 1.74 \times (HL) + 7.91 \quad \text{Equation 4.1}
\]

\[
r^2 = 0.972
\]

\[
(n = 83 \text{ observations of 8 individuals})
\]

where HL is head length (from the tip of the nose to the nuchal crest at the rear of the skull) in mm. This relationship was found to be most accurate for smaller young, i.e. for head-lengths between 40 and 70 mm. Beyond this size the variation between individuals became more marked (Figure 4.3).
4.2.4 Statistical Methods

Milk composition data from each year were pooled into age and weight (of young) classes and averaged. Age classes were 40 days wide from 100 to 420 days, and weight classes were 60 to 100, 101 to 200, 201 to 400 and thence every 400g to 3200g. Transformed \((\text{arcsin}(x)^{0.5})\) milk composition data from the four different koalas in 1990 and 1991 for which a full lactation's data were available, were compared by ANOVA for individual constituents, with year as one factor and period within the year (4 repeated measures) as a second factor. Due to the limited number of independent samples the statistical analysis of milk composition was limited, and the data are chiefly presented graphically.

4.3 RESULTS

4.3.1 Statistical comparison between years

There were no significant differences in milk composition between the two years. However, the two years' data have not been pooled because of interaction effects (age class X year, Table 4.1) which were significant or approached significance in all
constituents except carbohydrates. However, in the cases of carbohydrate and protein, the heterogeneous variances invalidated the test and thus differences could not be reliably detected.

Table 4.1 Comparison of milk composition between and within years.

<table>
<thead>
<tr>
<th>Component</th>
<th>Source</th>
<th>DF</th>
<th>F ratio</th>
<th>P</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solids</td>
<td>Between animals</td>
<td>1</td>
<td>0.666</td>
<td>0.451</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>3</td>
<td>2.439</td>
<td>0.105</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Age class X Year 3</td>
<td>4.011</td>
<td>0.028</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Lipid</td>
<td>Between animals</td>
<td>1</td>
<td>0.276</td>
<td>0.622</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>3</td>
<td>3.238</td>
<td>0.052</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Age class X Year 3</td>
<td>2.926</td>
<td>0.068</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Protein(^1)</td>
<td>Between animals</td>
<td>1</td>
<td>0.996</td>
<td>0.364</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Age class X Year 3</td>
<td>14.140</td>
<td>0.000</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>Carbohydrate(^1)</td>
<td>Between animals</td>
<td>1</td>
<td>0.314</td>
<td>0.599</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Age class X Year 3</td>
<td>166.175</td>
<td>0.000</td>
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<td>***</td>
</tr>
<tr>
<td>Energy</td>
<td>Between animals</td>
<td>1</td>
<td>0.357</td>
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<td>Age class X Year 3</td>
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<td>Age class X Year 3</td>
<td>2.883</td>
<td>0.071</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

* Statistically significant at P<0.05.
*** Statistically significant at P<0.001
\(^1\) Variances are heterogeneous (Cochran's test, P<0.05).

The interaction effects are graphically presented in Figure 4.4. The lipid content of late-stage (period 4) milk differed between the years (Figure 4.4 b), and this is reflected in the solids and energy contents (Figure 4.4 a and e). In 1990, the lipid, and subsequently solids and energy, content of the milk reached a peak in period 3 before falling to the late level (period 4). However, in 1991 lipid content did not peak in period 3, rather it fell then rose in the fourth period.

The highly significant interaction term for protein content was due to a lower level during the first period in 1991 than in 1990 (Figure 4.4 b), but could also have been partly an artifact of the heterogeneity of the variances.
Figure 4.4 Comparison of composition of koala's milk in 1990 and 1991 (means ± SD of 4 koalas in each year from the repeated measures ANOVA, section 4.3.1). a) Solids, b) Protein, c) Carbohydrate, d) Lipid, e) Energy.
4.3.2 Milk Composition

4.3.2.1 Total Solids, Lipid, Protein and Carbohydrate

The solids content of milk rose from 26.5 % (1991) at 130 days to a peak of around 33 % (33.8 % 1990, 32.2 % 1991) close to 200 days (Figure 4.5 a and b) and about 300g mass of young (Figure 4.6 a and b). After the initial peak the solids content of milk decreased to 26.3 % at 390 days and 2200g in 1990. The latest values in both years should be interpreted with caution as they come from only 2-3 samples; the majority of koalas had weaned the young earlier than 390 days. In 1991, the solids content dropped after the initial peak to 26-27 % at 960g and 280 days respectively before rising to a second peak of 31 % at 1700g and falling again (Figure 4.6 b).

Milk lipid content followed a similar pattern to that of solids, with peaks around 16 to 17 %. Much of the variation in solid content over the course of the lactation in both years was due to changing levels of lipid. It is worth noting that the secondary peak of lipid in 1991 raised the lipid, solids and energy content (Figures 4.6 a and b, and 4.10 a and b) up to the levels for the equivalent period in 1990.

Protein content of milk rose as lactation progressed in both years, from 5.5 % at 130 days and 84g mass of young to about 11 % at 390 days or 2000g.

Carbohydrate levels were greatest in the earliest samples, around 9 % at 130 days and 84g; they then dropped steadily over time (Figure 4.5) to about 1 % at 390 days. When plotted against the mass of the young (Figure 4.6), the fall in carbohydrate content was initially rapid and became progressively slower.

4.3.2.2 Composition of milk solids

In both years, lipid was the largest component of the total solids at all times except at the beginning and end of the sampling period. In the earliest samples, 130 days (Figure 4.7 b), both lipid and carbohydrate made up just over 30 % of the solids. As the proportion of carbohydrate fell, lipid rose to about 52 %, and fell again as the proportion of protein rose from about 20 % of the early samples to over 40 % of the latest samples (Figures 4.7 and 4.8).
Figure 4.5 Composition of koala milk by stage of lactation in a) 1990 and b) 1991 (means ± SE). PPE is permanent pouch exit.
Figure 4.6 Composition of koala milk by mass of the young in a) 1990 and b) 1991 (means ± SE). PPE is permanent pouch exit.
Figure 4.7 Composition of koala milk solids by age of the young in a) 1990 and b) 1991 (means ± SE). Sample sizes as in Figure 4.5. PPE is permanent pouch exit.
Figure 4.8 Composition of koala milk solids by mass of the young in a) 1990 and b) 1991 (means ± SE). Sample sizes as in Figure 4.6. PPE is permanent pouch exit.
4.3.2.3 Energy content

The pattern of changes in energy content of koala milk was similar, both within and between years, to that of changes in solids and lipid content. In 1990, the energy content of koala milk rose from 701 kJ.(100g)^{-1} at 164 days to a peak of 950 kJ.(100g)^{-1} at 236 days and then declined to 740 kJ.(100g)^{-1} at 390 days (Figures 4.9 a and 4.10 a). In 1991, the early pattern was similar, with a peak at 200 days, but there was a secondary peak in late lactation (Figures 4.9 b and 4.10 b).

Lipid provided over 50 % and up to 69 % of the energy of milk throughout lactation (Figures 4.11 and 4.12). Protein provided a rising proportion of milk energy, from around 20 % to 37 %, while carbohydrate contributed the least, falling from just over 20 % to 2 %.

4.3.2.4 Carbohydrate composition

Analysis of the carbohydrate composition by TLC showed that the carbohydrate fraction of koala milk is composed largely of oligo-saccharides in early lactation, with the appearance of a disaccharide, lactose, late in lactation (Figure 4.13). From 130 to 250 days, oligomers with the mobility of galactosyllactose to pentagalactosyllactose were present, as well as compounds that didn't migrate from the origin, or migrated very little (Figure 4.13). Some of these were probably larger oligo-saccharides (up to octa-galactosyllactose has been identified from tammar wallaby milk) or sialyl saccharides (Messer and Green, 1979; Marshall et al., 1990), and the compound remaining at the origin may be a glyco-protein (Marshall et al., 1990; M. Messer, pers. comm.). At 250 days, the time of pouch exit, the non-mobile compound decreased to very low levels, while lactose and an unidentified compound that was probably N-acetyl galactosamine appeared (M. Messer, pers. comm.). Total carbohydrate levels also decreased throughout lactation, so the quantities (as determined subjectively by the intensity of the spot) of the oligo-saccharides gradually declined over time (Figure 4.13).
Figure 4.9 Energy content of koala milk by age of the young in a) 1990 and b) 1991 (means ± SE). Sample sizes as in Figure 4.5. PPE is permanent pouch exit.
Figure 4.10 Energy content of koala milk by mass of the young in a) 1990 and b) 1991 (means ± SE). Sample sizes as in Figure 4.6. PPE is permanent pouch exit.
Figure 4.11 Relative contribution of lipid, protein and carbohydrate to the energy content of koala milk by age of the young in a) 1990 and b) 1991 (means ± SE). Sample sizes as in Figure 4.5. PPE is permanent pouch exit.
Figure 4.12 Relative contribution of lipid, protein and carbohydrate to the energy content of koala milk by mass of the young in a) 1990 and b) 1991 (means ± SE). Sample sizes as in Figure 4.6. PPE is permanent pouch exit.
Figure 4.13 Thin-layer chromatography of carbohydrates from koala milk throughout the period of lactation. PPE is permanent pouch exit. $S_1$ and $S_2$ are the standards. GluNAc is N-acetyl glucosamine (0.4 %), Glu is glucose (0.2 %), Gal is galactose (0.2 %), Gal-Lac is $3'$-galactosyllactose (0.3 %), Gal$_2$-Lac is $3',3''$-digalactosyllactose (0.4 %), Gal$_3$-Lac is $3',3'',3''$-trigalactosyllactose (0.4 %), Gal$_4$-Lac is $3',3'',3''',3''''$-tetragalactosyllactose and Gal$_5$-Lac is $3',3'',3''',3''''',3'''''$-pentagalactosyllactose.
4.4 DISCUSSION

4.4.1 Sampling Bias

Milk composition varies according to the stage which lactation is in, not only in marsupials (Green, 1984) but also many eutherians, especially at the extremes of the lactation period (Oftedal, 1984).

Milk composition changes over the time it accumulates in the gland (Brody, 1945; Oftedal, 1984), and according to previous milking history, diurnal rhythms and the proportion of milk stripped from the gland during sampling (Ling et al., 1961; Oftedal, 1984). Therefore the sampling regime should mimic normal suckling in that species. Often that information is unavailable, so milk should be allowed to accumulate for some minimum period of time (2 h in this study) and the gland stripped as fully as possible (Oftedal, 1984). The accumulation time should not be much longer than the normal inter-suckling interval or mammary involution might begin (Lascelles and Lee, 1978; Pitelka and Hamamotu, 1983). The high protein content of late lactation milk samples in the koala (Figures 4.5 and 4.6) may be due to involution of mammary alveolar cells as suckling decreased (Pitelka and Hamamotu, 1983), as was observed in ringtail possums (Munks et al., 1991). Use of large doses of oxytocin can result in lower carbohydrate levels in the milk (Peaker, 1978). These biases make it important that standard sampling procedures are determined for comparative studies.

In this study the sampling procedures, and analytical techniques, were as close as practicable to those suggested by Oftedal (1984) and similar to most others used in studies of marsupial milk composition, and so provide an accurate representation of the composition of koala milk, especially in comparison with other marsupials.

4.4.2 Comparison of koala milk with other marsupials

Green (1984) and Green and Merchant (1988) suggested that marsupials displayed only one lactational strategy, based on the similarity of the relative timing and patterns of changes in milk composition among species (Figure 4.1). Milk from early lactation is dilute though high in carbohydrates (mainly oligosaccharides), while milk in later lactation is concentrated, high in lipid and low in carbohydrate (mainly mono- and di-saccharides) (Green and Merchant, 1988).
The composition of koala milk is within the range of values found in other marsupials (Table 4.2). However, the composition and pattern of change in composition of koala milk throughout lactation differs from other marsupials in certain aspects. These are;

i) There is a decline in the solids content of koala milk at about pouch emergence, as in brushtail and ringtail possums (Cowan, 1989; Munks et al., 1991), but unlike most other marsupials (Figure 4.1; Green and Merchant, 1988), though the decline is not as large or as well defined as in the possums.

ii) Lipid is at a high level throughout lactation in the koala and provides most of the energy of milk (Figures 4.5 and 4.12), even in the earliest samples, again unlike most other marsupials (Figure 4.1; Green and Merchant, 1988). Lipid levels may have been low in phase 2a of lactation in the koala, but no samples could be collected from that phase in this study. Janssens and Ternouth (1987) speculated that the low levels of lipid and reliance on carbohydrate for energy during early lactation in other marsupials may indicate that the young have a limited capacity to digest or metabolize lipids, but the high concentrations of lipid in koala milk suggest that pouch young koalas can utilize lipids.

Carbohydrates and minerals are the major osmotic components of milk (Linzell and Peaker, 1971), which is constrained to osmolarity similar to that of maternal blood (Jenness and Sloan, 1970). The oligosaccharides characteristic of phase 2 marsupial milk increase the energy content of the carbohydrates in the milk far more, at a given osmotic pressure, than the mono- or disaccharides found in eutherian or marsupial phase 3 milk (Messer and Mossop, 1977; Messer and Green, 1979; Messer et al., 1987; Green and Merchant, 1988). In the tammar wallaby, and similarly in other marsupials, the length of carbohydrate chains increases till about day 180, when they are mainly oligosaccharides, and then decreases till day 280 when only monosaccharides such as glucose, galactose, N-acetylgalcosamine and N-acetylgalactosamine are present (Green and Merchant, 1988). In koalas, the pattern of change in carbohydrate structure is similar to that in the tammar wallaby, though the disaccharide lactose and some oligosaccharides, not monosaccharides, predominate after permanent pouch exit. Lactose, rather than monosaccharides, is also the major carbohydrate of phase 3 milk from ringtail and brushtail possums (Crisp et al., 1989a; Munks et al., 1991). Munks (1990) suggested that the disaccharide lactose allowed higher levels of carbohydrate in phase 3 milk of ringtails, supplementing the low energy content due to the low lipid levels. However, in koala milk, lipid levels are higher than in ringtail milk, precluding the necessity for energy supplementation.
Table 4.2 Milk composition in marsupials (% w/w).

<table>
<thead>
<tr>
<th>Species</th>
<th>Solids</th>
<th>Lipid</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>M</td>
<td>L</td>
<td>E</td>
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<tr>
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<td>26</td>
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<td>16</td>
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<td>35</td>
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<tr>
<td>Ringtail Possum</td>
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<td>25</td>
<td>13</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
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<td>28</td>
<td>18</td>
<td>9</td>
<td>5</td>
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<tr>
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<td>31</td>
<td>22</td>
<td>8</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Tammar wallaby</td>
<td>12</td>
<td>30</td>
<td>40</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Red-necked wallaby</td>
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<td>27</td>
<td>3</td>
<td>7</td>
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<tr>
<td>Tasmanian bettong</td>
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<td>25</td>
<td>40</td>
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<tr>
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<td>50</td>
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<td>40</td>
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<td>15</td>
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<tr>
<td>Eastern quoll</td>
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<td>34</td>
<td>4</td>
<td>15</td>
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<td>30</td>
<td>30</td>
<td>7</td>
<td>19</td>
<td>2</td>
</tr>
</tbody>
</table>

Changes in milk carbohydrate composition throughout lactation in brushtail possums are related to changes in the nature of the young's intestinal galactosidase enzymes (Crisp et al., 1989b); presumably similar changes take place in the gut of suckling koalas around the time of permanent pouch exit.

In the koala, as in the ringtail possum (Munks et al., 1991), there is no switch from carbohydrates to lipid as the major energy supply in milk at pouch exit. In other marsupials this is considered to be a consequence of rapidly rising energy requirements as the young become thermoregulatorily competent (Wallis and Maynes,
1973; Loh and Shield, 1977; Green, 1984; Janssens and Messer, 1988). In the koala, lipid is the most important source of energy in milk well before pouch exit, and remains so throughout lactation. In the ringtail possum, lipid levels are low throughout lactation, although they do rise slightly near the time of pouch exit. Munks (1990) suggested that the low lipid levels were a consequence of the extended weaning period in the possums, though if this were the case the koala would also be expected to have milk low in lipid.

The carbohydrate content of koala milk falls earlier than it does in the tammar wallaby, in which the decline in carbohydrate concentration is sudden (Figure 4.14), and usually occurs during the period of pouch emergence (Messer and Green, 1979). In koalas, carbohydrates fall steadily from well before the period of pouch emergence (Figures 4.5 and 4.14). The rise in lipid and fall in carbohydrate content of milk usually accompanies the change in diet from milk to the adult diet that begins at pouch exit. In the foregut-fermenting macropodids these changes may be necessary to allow establishment of gut flora (Janssens and Ternouth, 1987), and are accompanied by metabolic changes that allow the young to utilize their new energy source (Janssens and Ternouth, 1987; Janssens and Messer, 1988; Janssens and Rogers, 1989). In tammar wallabies, activities of gluconeogenic enzymes, especially Phospho-enol-pyruvate carboxy kinase (PEPCK), increase several fold at pouch exit, while the glycolytic enzyme Pyruvate kinase (PK) decreases (Wilkes and Janssens, 1986). This allows the developing tammar wallaby to meet its requirements for glucose from gluconeogenesis.

The emphasis on lipid as the major energy source throughout lactation in the koala indicates that the young must be able to utilize lipid as an energy source. Activity of gluconeogenic enzymes in developing koalas is unknown, but may not be the same as in tammars. Adult koalas are hindgut fermenters (Hume, 1982) and rely on unfermented cell contents for 90% of their energy (Cork et al., 1983), so consumption of foliage would probably provide dietary carbohydrates to young koalas even though the milk carbohydrate was declining.

Lipid levels in koala milk were higher than in the other marsupial folivores (Table 4.2), despite the similar diet, and closer to the levels in the wombat. This may indicate that milk composition is influenced by phylogeny, as in eutherians (Oftedal, 1984). It may also be an artifact of the use of free-living koalas in this study, as opposed to the captive animals used in most other studies. Munks et al. (1991) found that late lactation milk from wild ringtail possums was higher in lipid and lower in protein than that from captive animals.
4.4.3 Differences between the years: effects of diet?

The patterns of changes in protein and carbohydrate levels of koala milk were similar in the two years sampled (Figures 4.5 and 4.6). In the comparison between years (Section 4.3.1) protein was lower in the early samples from 1991 than from 1990 (Figure 4.4 b), although that comparison was based on only eight koalas. Protein concentration was not low in early samples from 1991, based on visual examination of the full data set (Figure 4.5). The variation in solids and energy within and between years follows the pattern of variation in lipid levels (Figures 4.5, 4.6 and 4.10), so it seems that while protein and carbohydrate content of koala milk follow an orderly pattern, the lipid and consequently solids and energy levels are far more labile.

The years of sampling of milk in this study, 1990 and 1991, differed in rainfall and in the availability of new foliar growth in the spring and summer, during phase 3 of the koala's lactation. In 1990, rainfall was near average (Forestry Commission, 1987) and new foliage was available throughout phase 3 of lactation, but in 1991 during phase 3 of lactation, rainfall was low and new foliage was not available until late November (Figure 8.1).

The rise in milk lipid at 320 days of lactation in 1991 (Figure 4.5 b) corresponded to the flush of new foliage in November, and took the lipid, solids and energy concentration of the milk up to approximately the same levels found in 1990 for that stage of lactation. (The last point in 1991 (Figure 4.5 b) was from 2 samples only, and may not be representative of the population). There were similar rises in the proportion of lipid in milk solids and the contribution of lipid to the energy of milk during 1991 (Figures 4.8 b and 4.12 b).

The main difference between the years was that lipid concentration, and consequently solids and energy content, were comparatively low in 1991 before new foliage became available (Figure 8.1). New Eucalyptus leaves, or "tips", are generally selected by koalas over old leaves (Ullrey et al., 1981; Hindell, 1984; Pahl and Hume, 1990; Hume and Esson, 1993) and have lower fibre and higher nitrogen concentrations (Ullrey et al., 1981; Cork, 1984; Cork and Pahl, 1984; Kavanagh and Lambert, 1990; Hume and Esson, 1993; This study, Chapter 8), so the differences in milk composition may have been due to different dietary quality in the two years (ARC, 1980; Forsum and Lonnerdal, 1980; Prentice, 1980; Thomas and Chamberlain, 1984; Thomas and Martin, 1988).
The effects of dietary lipid on secretion of milk are complex and depend on the levels of lipid in the diet and its fatty acid composition (Thomas and Martin, 1988). A rise in dietary lipid causes milk lipid and protein levels to rise in cattle if the diet was originally deficient in lipid (Banks et al., 1976) but can cause a depression in milk lipids if the diet is high in lipid (Steele and Moore, 1968). The mechanism of depression of milk lipids by dietary lipid may be interference with rumen function (Thomas and Martin, 1988) however this is unlikely in the hindgut fermenting koala. A rise in dietary lipid has also been shown to depress milk protein concentration in cattle, but not protein yield, suggesting that the rate of lactose synthesis increased, leading to a greater volume of dilute milk (Bines et al., 1978; Thomas and Martin, 1988). Milk lipids in humans are positively affected by increases in dietary lipids (Prentice, 1980).

Rises in dietary protein can raise milk lipid concentration (Orskov et al., 1977; Oldham et al., 1984) or yield (Clarke, 1975), possibly by increasing the mobilization of adipose tissue in cattle (Whitelaw et al., 1987). Milk protein levels can be positively affected by rises in dietary protein (Forsum and Lonnerdal, 1980; Thomas and Martin, 1988), although the effect is dependent on the amino acid composition (Schwab et al., 1976; Rogers et al., 1987).

Increases in dietary carbohydrate can reduce milk lipid concentration in cattle (Sutton, 1985; McRae et al., 1988), possibly by increasing insulin levels (Palmquist and Moser, 1981; Hart, 1983) and promoting deposition of energy in adipose tissue rather than export in milk (Flatt et al., 1969; Orskov et al., 1969; Thomas and Martin, 1988), although experiments attempting to mimic the effect by administration of insulin have failed (Thomas and Martin, 1988).

Water restriction can result in an increase in milk solids in some rodents (Baverstock et al., 1976), and a decrease in milk solids in camels, humans, cattle and possibly harp seals (Yagil and Etzion, 1980; Lavigne et al., 1982; Yagil et al., 1986). Yagil et al. (1986) suggest that the mechanism for dilution of milk in response to water stress occurs via the cross reactivity of oxytocin (the milk let-down hormone) and ADH (anti-diuretic hormone) (Lawrence, 1980), and ensures that the water requirements of the young are met. However, koala milk was not diluted or concentrated in response to water stress in 1991, as levels of carbohydrate and protein were similar to those in 1990.

Differences in dietary quality and composition between years in this study, particularly in the available protein and lipid content due to the different availability of new foliage, may have caused the observed differences in the patterns of changing
milk composition. However, after pouch exit, when most of the differences between years were apparent, the milk intake of the young declines (Dove and Cork, 1989; Chapter 5), so the significance of small changes in milk composition may be very small. In the later stages of lactation milk may function as a protein and lipid supplement for young koalas largely dependent on foliage. The next chapter considers the intake of milk, the nutrition of the young and the magnitude of the demands of lactation in koalas.
CHAPTER FIVE

MILK PRODUCTION AND GROWTH AND NUTRITION OF THE YOUNG

5.1 INTRODUCTION

Lactation, especially at its peak, is energetically demanding for the mother, leading to large increases in her energy requirements (Table 1.2). Because the energy requirements of lactation are met largely by increased nutrient intake in most mammals, limits to intake may be expected to limit the allocation of energy to reproduction (Kenagy et al., 1990). The koala is subject to allometric constraints on its ability to extract nutrients from its diet (i.e. a high ratio of energetic requirements to gut capacity), and is thought to exist on a fine energetic balance (Cork and Sanson, 1990). It is likely that those allometric constraints and fine energetic balance are reflected in the pattern of allocation of energy to reproduction by koalas. The rate of milk-energy transfer to the young by female koalas is therefore expected to be low in comparison with other mammals. This is in accordance with the slow growth rate of juvenile koalas (Russell, 1982a; Martin and Handasyde, 1990) and the long duration of lactation (Russell, 1982a).

Milk production has been previously measured in only three species of marsupial, the tammar wallaby, *Macropus eugenii* (Green and Newgrain, 1979; Cork and Dove, 1989; Dove and Cork, 1989), the northern brown bandicoot, *Isoodon macrourus* (Merchant, 1990) and the common ringtail possum, *Pseudocheirus peregrinus* (Munks, 1990), so there are insufficient data to make allometric predictions of the lactational output, as has been done with eutherians (Oftedal, 1984, 1985). Both of the marsupial herbivores for which lactational output has been measured are small and eat a fibrous diet (and so like the koala are subject to high energy requirements relative to their ability to extract nutrients from their diet) and their lactational output is low compared with that expected for eutherians of the same mass (Oftedal, 1984). Peak milk-energy output by *M. eugenii* was 700 kJ.d⁻¹ (Cork and Dove, 1989) and that of *P. peregrinus* (Munks, 1990) was 156 kJ.d⁻¹, only 43 % and 26 % respectively of that expected for a similar sized eutherian. Milk-energy output by the koala could be expected to be similarly low.

Koala milk lipid content was greater in 1990 than 1991, probably due to the relative quality of foliage available in the two years (Chapter 4). Milk yield is also affected
by diet quality (Thomas and Martin, 1988), so it is possible that milk-energy output was greater in 1990 than 1991.

Juvenile koalas, like their mothers, are subject to allometric limits to their abilities to extract their nutrient requirements from foliage. If the size of young at weaning represents the minimum mass for survival in the adult niche (Millar, 1977), then the relationship between the developing energy requirements and ability to extract nutrients from the adult diet are likely to set a lower limit to the energy input by the mother to ensure successful reproduction.

This chapter describes the energetics and nutrition of young koalas, and hence the magnitude of the energetic demands of lactation on the mother.

5.2 MATERIALS AND METHODS

5.2.1 Milk production

Milk production was measured by a double isotope method (Holleman et al., 1975) during the periods of water intake measurement (Table 3.4). In this method milk production is measured as the input of maternal water to the young, assuming that all milk produced was consumed by the young. Measurement of the water intake of adult female koalas is described in Section 6.2.1.2. Water intake was measured similarly in the young, though using deuterium oxide ($^2$H$_2$O) at a dose rate of 2 ml.kg$^{-1}$, instead of $^3$H$_2$O, as the tracer (Schoeller et al., 1986). All the other procedures were the same as described in Chapter 6 (Section 6.2.1.2) with the additional conditions that water intake of the young was measured over the same period as that of the mother, and the blood volume sampled was 1-2 ml (depending on the mass of the young).

In some of the small juveniles (< 300 g), where intra-venous dosing of the isotope was impracticable, the dose was delivered intra-peritoneally. On nine occasions, again with very small young, the blood samples obtained were insufficient for the determination of equilibrium concentrations of $^2$H$_2$O. In these cases, and where isotope was lost during injection, the body water pool was estimated from a regression of pool size on the masses of other young (Figure 5.1, Equation 5.1), and the equilibrium concentration of $^2$H$_2$O was estimated as the dose divided by the pool size.

Blood water samples were prepared as described in Section 6.2.1.3; those of the mothers were analysed for $^3$H$_2$O content (by Liquid-Scintillation Spectroscopy, Section 6.2.1.3), and those of the young for $^2$H$_2$O content by Infra-red
Spectrophotometry (Byers 1979, Dove and Freer, 1979), as well as for $^3$H$_2$O as previously described.

In the analysis of $^2$H$_2$O, 0.6 ml aliquots of the purified blood water were injected into the fixed calcium fluoride cell of the Miran 1A IR spectrophotometer and the absorbance at 4 μm read at 2 min. after injection. This was to standardise the procedure, as the cell was not temperature controlled and absorbance decreased over time. Standards were prepared from certified $^2$H$_2$O enriched water (99 %) diluted by mass with distilled water, and used to prepare a standard curve analysed with each batch of samples. Absorbance was linearly related to $^2$H$_2$O concentration over the 0 to 4000 ppm range. The relationship was determined by least squares linear regression (Wilkinson, 1989).

Milk-water intake by the young was calculated from the accumulation of the maternal tracer ($^3$H$_2$O) in the young by the equations of Holleman et al. (1975), with correction for changing body-water pool size of the young (Dove and Freer, 1979). These equations (Holleman et al., 1975) correct for the loss of $^3$H$_2$O from the young using the turnover of $^2$H$_2$O measured concurrently. The data were based on 32 measurements in 10 individuals in 1990 and 37 measurements in 10 individuals in 1991, three of which were common to both years.

Foliage intake by young was estimated by subtracting milk and metabolic water from total water intake measured by the turnover of $^2$H$_2$O and adjusting for the solids content of the foliage (as in Section 6.2.1.5).

Metabolic water production was estimated in those juveniles in which field metabolic rate (FMR) was measured, using conversion factors for metabolic water production from lipid, protein and carbohydrate (Withers, 1992). The relationship between metabolic water production and juvenile mass was derived by linear least squares regression, and used to estimate the metabolic water production of the young during the other measurement periods.

### 5.2.2 Energetics

#### 5.2.2.1 Field metabolic rate

Field metabolic rates of juvenile koalas were measured during three periods (Table 3.4) at four stages of development: just after pouch exit (8 months), between pouch exit and complete weaning (10.5 months), just after completion of weaning (13 months) and at about 20 months.
Field metabolic rates were measured using doubly-labelled water as described in Section 6.2.1.2, except that $^2$H$_2$O was substituted for $^3$H$_2$O, at a dose rate of 2 ml.kg$^{-1}$.

Carbon dioxide production (ml.h$^{-1}$) was converted to field metabolic rate (kJ.d$^{-1}$) using published conversion factors (Gessaman and Nagy, 1988). At 260 days of age the young were not consuming significant quantities of foliage, so the respiratory exchange coefficient and conversion factors were calculated from the composition of milk at that time (Figure 4.11). I have assumed that no protein is catabolised, so most of the energy in milk is in the form of lipid, giving a conversion factor of 27.18 kJ.l$^{-1}$ CO$_2$. The conversion factor associated with the catabolism of foliage at 400 days and older was 21.2 kJ.l$^{-1}$ CO$_2$ (Foley et al., 1990, as for the adults in Chapter 6). At 320 days, milk (with 93% of the energy as lipid, Figure 4.5 b) contributed about 30% of the juveniles' water intake (Figure 5.5). I have therefore assumed that milk supplied 30% and foliage 70% of the energy; this yields a conversion factor of 23.15 kJ.l$^{-1}$ CO$_2$.

5.2.2.2 Resting metabolic rate

Resting metabolic rates (RMR) were measured in three captive juvenile koalas (that were not fasted), just after the time of pouch exit. The measurements were made by flow-through respirometry as described for adult females in Section 6.2.2, except that a lower flow rate, 2 l.min$^{-1}$ was used. The respiratory exchange coefficient measured in the fed young was similar to that in the fasted adults. As most energy intake at this stage was from milk-lipid (Figs 4.5 b and 5.5) I used the factor of 19.8 kJ.l$^{-1}$O$_2$ to convert oxygen consumption to metabolic rate (Gessaman and Nagy, 1988).

5.2.2.3 Maternal energy requirements for lactation

The metabolic energy requirements of lactation in koalas was calculated from the intake of milk-energy by the young. Milk production was assumed to equal consumption, and the efficiency of conversion of metabolic energy to milk-energy was assumed to be 65% (ARC, 1980). Milk-energy output was expressed in terms of energy per unit metabolic body mass (kJ.kg$^{-0.75}$, Oftedal, 1984) and temporal stage of lactation as metabolic time (d.kg$^{-0.25}$, Calder, 1984) for comparison with other studies.

The total metabolic energy requirement for lactation was estimated as the area of trapezia under the curves in Figure 5.12.
5.2.3 Growth

Growth of dependent young was compared between years using average sizes and masses of young at around pouch exit (260 days), 300 days and close to completion of weaning, 350 days. Means were compared using Student's T-test at each of the three times (Wilkinson, 1989). Gompertz growth curves were not fitted to the mass changes of the young, despite the value of the Gompertz equation for describing marsupial growth (Lee and Cockburn, 1985), as there were insufficient longitudinal data for each individual to be able to accurately determine the parameters.

5.2.4 Statistical

Intake of milk components by juvenile koalas were pooled by age classes of the juveniles and also by mass classes, as Dove and Cork (1989) found a strong relationship of milk intake with juvenile mass. Because there were significant differences between years in the intake of some milk components by young koalas, data were not pooled between the years but are presented separately.

Milk intake was compared between the two years using a repeated measures ANOVA (Wilkinson, 1989) with year as one factor and age or mass class of the young as the other. A separate set of female koalas from each year were selected from the full database and longitudinal data from their offspring used for the repeated measure of intake by age or mass classes. A total of seven koalas was used in this analysis, three in 1990 and four in 1991. These were the only animals with measures in each of the 4 age/mass classes used that were not repeated between years. Homogeneity of variances was tested using Bartlett's test (Wilkinson, 1989).

Differences in milk intake between the years were also tested at 260 days age of young, at or close to the peak of lactation in both years, by t-tests, including individuals that were represented in both years data in only one of the years selected randomly.

5.3 RESULTS

5.3.1 Comparison between years

Intake of some milk components differed between years (Table 5.1); this is shown most clearly in the data pooled by mass classes. Intake of milk-lipid was significantly greater in 1990 than in 1991 (Figure 5.8), while the difference in milk-solids intake (Figure 5.7) between the two years approached significance (P=0.062, data pooled by mass). Consequently the difference in milk-energy intake between the years,
Table 5.1 Comparison of milk and water intake of juvenile koalas in 1990 and 1991

<table>
<thead>
<tr>
<th>Component</th>
<th>Source</th>
<th>DF</th>
<th>Probability&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Significance&lt;sup&gt;4&lt;/sup&gt;</th>
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<td></td>
<td></td>
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<td>Mass&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total water</td>
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</tr>
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<td></td>
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<td>0.872</td>
<td>0.882</td>
</tr>
<tr>
<td>Solids</td>
<td>Year</td>
<td>1</td>
<td>0.091</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>Class</td>
<td>3</td>
<td>0.458</td>
<td>0.133</td>
</tr>
<tr>
<td></td>
<td>Class*Year</td>
<td>3</td>
<td>0.334</td>
<td>0.828</td>
</tr>
<tr>
<td>Lipid</td>
<td>Year</td>
<td>1</td>
<td>0.050</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>Class</td>
<td>3</td>
<td>0.632</td>
<td>0.127</td>
</tr>
<tr>
<td></td>
<td>Class*Year</td>
<td>3</td>
<td>0.121</td>
<td>0.721</td>
</tr>
<tr>
<td>Protein</td>
<td>Year</td>
<td>1</td>
<td>0.244</td>
<td>0.160</td>
</tr>
<tr>
<td></td>
<td>Class</td>
<td>3</td>
<td>0.372</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td>Class*Year</td>
<td>3</td>
<td>0.714</td>
<td>0.826</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Year</td>
<td>1</td>
<td>0.190</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td>Class</td>
<td>3</td>
<td>0.008</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Class*Year</td>
<td>3</td>
<td>0.290</td>
<td>0.498</td>
</tr>
<tr>
<td>Energy</td>
<td>Year</td>
<td>1</td>
<td>0.067</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>Class</td>
<td>3</td>
<td>0.591</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td>Class*Year</td>
<td>3</td>
<td>0.233</td>
<td>0.782</td>
</tr>
</tbody>
</table>

<sup>1</sup> Probability of a difference in intake of the component being due to chance alone.
<sup>2</sup> Data pooled by age of the young.
<sup>3</sup> Data pooled by mass of the young.
<sup>4</sup> * P < 0.05, ** P < 0.01, *** P < 0.001
DF = degrees of freedom.
achieved (P=0.045, data pooled by mass) or approached (P=0.067, data pooled by age classes) significance. Total water intake of juvenile koalas was greater in 1990 than 1991 (Table 5.1), but milk-water intake did not differ between the years. Thus intake of water from food must have been greater in 1990.

Table 5.1a. Comparison of milk and water intake of 260 day old juvenile koalas in 1990 and 1991 (means ± SD).

<table>
<thead>
<tr>
<th>Component</th>
<th>1990 (n = 5)</th>
<th>1991 (n = 5)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total water (g.d⁻¹)</td>
<td>51.6 ± 20.8</td>
<td>27.6 ± 12.5</td>
<td>0.056 ns</td>
</tr>
<tr>
<td>Milk-water (g.d⁻¹)</td>
<td>43.3 ± 11.1</td>
<td>26.9 ± 6.5</td>
<td>0.022 *</td>
</tr>
<tr>
<td>Milk-water: Total water (%)</td>
<td>93 ± 29</td>
<td>103 ± 17</td>
<td>0.491 ns</td>
</tr>
<tr>
<td>Milk- total (g.d⁻¹)</td>
<td>62.3 ± 15.6</td>
<td>38.4 ± 7.3</td>
<td>0.014 *</td>
</tr>
<tr>
<td>Solids (g.d⁻¹)</td>
<td>19 ± 5.3</td>
<td>11.5 ± 1.3</td>
<td>0.014 *</td>
</tr>
<tr>
<td>Lipid (g.d⁻¹)</td>
<td>9.5 ± 3.3</td>
<td>5.4 ± 1.2</td>
<td>0.030 *</td>
</tr>
<tr>
<td>Protein (g.d⁻¹)</td>
<td>4.9 ± 1.4</td>
<td>3.4 ± 0.7</td>
<td>0.058 ns</td>
</tr>
<tr>
<td>Carbohydrate (g.d⁻¹)</td>
<td>3.1 ± 1.4</td>
<td>1.7 ± 0.4</td>
<td>0.051 ns</td>
</tr>
<tr>
<td>Energy (kJ.d⁻¹)</td>
<td>534 ± 167</td>
<td>315 ± 44</td>
<td>0.022 *</td>
</tr>
</tbody>
</table>

P is the probability of the difference between the means being due to chance alone.
* indicates statistical significance at P = 0.05
ns indicates "not statistically significant".
Total milk intake, the ratio of total water to milk-water intake, and protein and carbohydrate intakes did not differ between the years (Table 5.1). Total water, total water: milk-water and carbohydrate intakes changed significantly, in a linear or approximately linear fashion within a year (Table 5.1), but the more complex (higher order) changes in intake of most of the other components are best seen graphically (Figures 5.3 to 5.11). However, single degree-of-freedom tests showed that changes in milk-water and total milk intake conformed to cubic polynomials (P=0.027 and 0.043 respectively, pooled by mass classes).

### 5.3.2 Water intake of the young

The body water space of juvenile koalas increased linearly with body mass (Figure 5.1):

\[
\text{Body water (g)} = 0.784 \times \text{Mass (g)} - 22.590 \\
\text{Equation 5.1}
\]

\[
\text{r}^2 = 0.985, \ P < 0.001 \\
(n = 60 \text{ measures of 20 individuals})
\]

The water intake of the young was linearly related to body mass, but exponentially related to age (Figure 5.2).
Figure 5.2 Water intake of juvenile koalas according to their a) age, and b) mass (32 measurements of 10 koalas in 1990, 37 measurements of 10 koalas in 1991).

Regressions

(i) Intake (g.d\(^{-1}\)) = 1.92 \times 10^{0.0054} \text{ Age (d)} \quad r^2 = 0.835

(ii) Intake (g.d\(^{-1}\)) = 1.34 \times 10^{0.0052} \text{ Age (d)} \quad r^2 = 0.938

(iii) Intake (g.d\(^{-1}\)) = 0.08 \times \text{ Mass (g)} + 0.98 \quad r^2 = 0.922

(iv) Intake (g.d\(^{-1}\)) = 0.073 \times \text{ Mass (g)} - 14.8 \quad r^2 = 0.952
As found in the limited inter-year comparison above (Table 5.1) the water intake of juvenile koalas was greater in 1990 than at an equivalent stage in 1991.

5.3.3 Milk intake of juvenile koalas

5.3.3.1 Milk-water intake

Milk-water intake rose throughout pouch life to a peak at or just after pouch exit (Figures 5.3 and 5.4). There was a significant difference between years in peak milk-water intake (Table 5.1a), with greater intake by the young in 1990 than 1991 (P = 0.022). Peak milk-water intake was about 43 g.d⁻¹ in 1990 and 33-36 g.d⁻¹ in 1991 at 250-300 days (Figure 5.3) or 700-1000 g (Figure 5.4).

After pouch exit (around 250 days or 500-700 g), the ratio of milk-water intake to total water intake decreased until weaning was complete, around 400 days at a mass of 2.5 to 3 kg (Figure 5.5).

5.3.3.2 Total milk and milk-solids intake

Total milk (Figure 5.6), and milk-solids intake showed the same pattern as milk-water intake (Figure 5.7). Peak intake in 1990 was greater than at the same stage in 1991 (Table 5.1a, P = 0.014 for both components). Milk-solids intake peaked at 18-19 g.d⁻¹ at 260 days and 740 g in 1990, and at 12-13 g.d⁻¹ at 260-300 days and 800 g in 1991 (Figure 5.7).

5.3.3.3 Milk-lipid, -carbohydrate and -protein intake

Milk-lipid intake showed the same pattern of changes as milk-water intake, and it was significantly greater in 1990 than 1991 (Table 5.1a; Figure 5.8). In 1990, the peak of lipid intake (9-10 g.d⁻¹ at 260 days, 500-1000 g) was also earlier than in 1991 (5-6 g.d⁻¹ at 300-340 days, 500-1200 g).

Carbohydrate intake peaked at 260 days or 700-800 g (Figure 5.9), and then fell rapidly as milk intake and the carbohydrate content of that milk fell (Figures 5.7 and 4.5). There was also a trend, although not significant, toward higher intake in 1990 (peak 2.9 g.d⁻¹) than in 1991 (peak 1.8 g.d⁻¹) (Table 5.1a).

Milk-protein intake peaked slightly later than the other components, at 300 days (Figure 5.10) and remained relatively high until just before weaning because of the increasing protein content of the milk (Figure 4.5). Again there was a trend toward higher milk-protein intake in 1990 than 1991 (Table 5.1).
Figure 5.3 Milk-water and total water intake of juvenile koalas by the age of the young in a) 1990 and b) 1991 (means ± SE). PPE is permanent pouch exit. The sample sizes are shown above the points (32 measurements of 10 koalas in 1990, 37 measurements of 10 koalas in 1991).
Figure 5.4 Milk-water and total water intake of juvenile koalas by the mass of the young in a) 1990 and b) 1991 (means ± SE). PPE is permanent pouch exit. The sample sizes are shown above the points (32 measurements of 10 koalas in 1990, 37 measurements of 10 koalas in 1991).
Figure 5.5 The proportion of total water intake by juvenile koalas that is taken in as preformed water in milk according to a) age and b) mass of the young (means ± SE). PPE is permanent pouch exit. Sample sizes for a) are as in Figs 5.3a and 5.3b for 1990 and 1991 respectively. Sample sizes for b) are as in Figs 5.4a and 5.4b for 1990 and 1991 respectively (32 measurements of 10 koalas in 1990, 37 measurements of 10 koalas in 1991).
Figure 5.6 Intake of milk by juvenile koalas according to a) age and b) mass of the young (means ± SE). PPE is permanent pouch exit. Sample sizes for a) are as in Figs 5.3a and 5.3b for 1990 and 1991 respectively. Sample sizes for b) are as in Figs 5.4a and 5.4b for 1990 and 1991 respectively (32 measurements of 10 koalas in 1990, 37 measurements of 10 koalas in 1991).
Figure 5.7 Intake of milk-solids by juvenile koalas according to a) age and b) mass of the young (means ± SE). PPE is permanent pouch exit. Sample sizes for a) are as in Figs 5.3a and 5.3b for 1990 and 1991 respectively. Sample sizes for b) are as in Figs 5.4a and 5.4b for 1990 and 1991 respectively (32 measurements of 10 koalas in 1990, 37 measurements of 10 koalas in 1991).
Figure 5.8 Intake of milk-lipids by juvenile koalas according to a) age and b) mass of the young (means ± SE). PPE is permanent pouch exit. Sample sizes for a) are as in Figs 5.3a and 5.3b for 1990 and 1991 respectively. Sample sizes for b) are as in Figs 5.4a and 5.4b for 1990 and 1991 respectively (32 measurements of 10 koalas in 1990, 37 measurements of 10 koalas in 1991).
Figure 5.9 Intake of milk-carbohydrates by juvenile koalas according to a) age and b) mass of the young (means ± SE). PPE is permanent pouch exit. Sample sizes for a) are as in Figs 5.3a and 5.3b for 1990 and 1991 respectively. Sample sizes for b) are as in Figs 5.4a and 5.4b for 1990 and 1991 respectively (32 measurements of 10 koalas in 1990, 37 measurements of 10 koalas in 1991).
Figure 5.10 Intake of milk-protein by juvenile koalas according to a) age and b) mass of the young (means ± SE). PPE is permanent pouch exit. Sample sizes for a) are as in Figs 5.3a and 5.3b for 1990 and 1991 respectively. Sample sizes for b) are as in Figs 5.4a and 5.4b for 1990 and 1991 respectively (32 measurements of 10 koalas in 1990, 37 measurements of 10 koalas in 1991).
Figure 5.11 Intake of milk-energy by juvenile koalas according to a) age and b) mass of the young (means ± SE). PPE is permanent pouch exit. Sample sizes for a) are as in Figs 5.3a and 5.3b for 1990 and 1991 respectively. Sample sizes for b) are as in Figs 5.4a and 5.4b for 1990 and 1991 respectively (32 measurements of 10 koalas in 1990, 37 measurements of 10 koalas in 1991).
5.3.3.4 Milk-energy intake

The peak milk-energy intake of young koalas in 1990 was 500 and 540 kJ.d⁻¹ at 740 g and 260 days respectively (Figure 5.11a). The peak in 1991 was lower (Table 5.1, 5.1a) and later; around 350 kJ.d⁻¹ at 300 days and 800 g (Figure 5.11b).

5.3.3.5 Maternal energy requirements for lactation

The peak daily metabolic energy requirements for lactation were 835 kJ.d⁻¹ at 260 days (188 kJ.kg⁻0.75.d⁻¹ at 150 d.kg⁻0.25) and 514 kJ.d⁻¹ at 300 days (153 kJ.kg⁻0.75.d⁻¹ at 207 d.kg⁻0.25) in 1990 and 1991 respectively (Figure 5.12). The total metabolic energy requirement for reproduction by koalas in 1990 was 53% greater than in 1991 (29.0 and 18.9 MJ.kg⁻¹ respectively). The total requirements are likely to have been overestimates if the early requirements for reproduction followed an exponential rather than linear increase.

Figure 5.12 The metabolizable energy (ME) requirements for reproduction in female koalas. Absence of data indicated by dotted lines (32 measurements of 10 koalas in 1990, 37 measurements of 10 koalas in 1991).

5.3.4 Foliage intake

Juvenile koalas started to consume foliage after pouch exit at about 260 days of age and 700-800 g body mass (Figure 5.13a and b). The intake of foliage was similar in the two years.
Figure 5.13 Intake of foliage dry matter by juvenile koalas according to a) age and b) mass of the young (means ± SE). PPE is permanent pouch exit. Sample sizes for a) are as in Figs 5.3a and 5.3b for 1990 and 1991 respectively. Sample sizes for b) are as in Figs 5.4a and 5.4b for 1990 and 1991 respectively (32 measurements of 10 koalas in 1990, 37 measurements of 10 koalas in 1991).
5.3.5 Energetics

5.3.5.1 Field metabolic rate

The FMR of juvenile koalas was linearly related to body mass (Figure 5.14; Equation 5.2):

\[
\text{FMR (kJ.d}^{-1}) = 0.247 \text{ Mass (g) } + 149.1 \\
\text{Equation 5.2}
\]

\[r^2 = 0.947, P < 0.001\]

(22 observations of 7 koalas)

Metabolic water production in the young was also linearly related to their body mass (Figure 5.15, Equation 5.3):
Figure 5.15 Metabolic water production by juvenile koalas.

Met. water (g.d\(^{-1}\)) = 0.0075 Mass (g) + 4.32

\[ r^2 = 0.948, P < 0.001 \]

(22 observations of 7 koalas)

### 5.3.5.2 Resting metabolic rate

The resting metabolic rate (RMR) of the captive juvenile koalas was 311 ± 25 kJ.d\(^{-1}\) or 244 ± 26 kJ.kg\(^{-0.737}\).d\(^{-1}\) (Table 5.2).

Table 5.2 Resting metabolic rate in fed juvenile koalas.

<table>
<thead>
<tr>
<th>Koala</th>
<th>Mass (g)</th>
<th>O(_2) consumption (ml.g(^{-1}).h(^{-1}))</th>
<th>RMR (kJ.d(^{-1}))</th>
<th>RMR (kJ.kg(^{-0.737}).d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>5J</td>
<td>1631</td>
<td>0.438</td>
<td>340</td>
<td>237</td>
</tr>
<tr>
<td>6J</td>
<td>1443</td>
<td>0.424</td>
<td>291</td>
<td>222</td>
</tr>
<tr>
<td>7J</td>
<td>1153</td>
<td>0.553</td>
<td>303</td>
<td>273</td>
</tr>
<tr>
<td>Mean</td>
<td>1409</td>
<td>0.472</td>
<td>311</td>
<td>244</td>
</tr>
<tr>
<td>SD</td>
<td>241</td>
<td>0.071</td>
<td>25</td>
<td>26</td>
</tr>
</tbody>
</table>
5.3.6 Growth of young

There were no differences between 1990 and 1991 in the size or mass of dependent juvenile koalas at a given age (Table 5.3).

Table 5.3 Size of dependent juvenile koalas at three ages (means, SD in parentheses).

<table>
<thead>
<tr>
<th></th>
<th>1990</th>
<th>1991</th>
<th>p1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (days)</td>
<td>258</td>
<td>259</td>
<td>0.732</td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass (g)</td>
<td>636</td>
<td>619</td>
<td>0.772</td>
</tr>
<tr>
<td>(115)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headlength (mm)</td>
<td>74.1</td>
<td>74.2</td>
<td>0.963</td>
</tr>
<tr>
<td>(1.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bodylength(^2) (mm)</td>
<td>228</td>
<td>231</td>
<td>0.823</td>
</tr>
<tr>
<td>(23)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (days)</td>
<td>299</td>
<td>296</td>
<td>0.386</td>
</tr>
<tr>
<td>(7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass (g)</td>
<td>1051</td>
<td>948</td>
<td>0.231</td>
</tr>
<tr>
<td>(159)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Headlength (mm)</td>
<td>83.0</td>
<td>80.5</td>
<td>0.172</td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bodylength (mm)</td>
<td>284</td>
<td>266</td>
<td>0.091</td>
</tr>
<tr>
<td>(20)</td>
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<td></td>
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</tr>
<tr>
<td>Age 3 (days)</td>
<td>349</td>
<td>354</td>
<td>0.205</td>
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<tr>
<td>(6)</td>
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<tr>
<td>Mass (g)</td>
<td>1788</td>
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<td>(242)</td>
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<tr>
<td>Headlength (mm)</td>
<td>91.8</td>
<td>92.0</td>
<td>0.922</td>
</tr>
<tr>
<td>(3.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bodylength (mm)</td>
<td>349</td>
<td>335</td>
<td>0.231</td>
</tr>
<tr>
<td>(19)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Probability of a difference by Student's T-test.
2 Bodylength from the base of the tail to the occipital crest at the base of the skull.

5.4 DISCUSSION

5.4.1 Errors in measurement of milk production

Holleman et al. (1975) validated the double isotope method of measuring milk intake, using bottle-fed calves, and found it to be accurate to within 2.5% of the true value. As with the measurement of water intake by adult koalas, the measurement of milk intake by isotopic techniques is associated with a number of conditions and assumptions, violations of which lead to errors in calculated fluxes (Section 6.4.3.1; Lifson and McClintock, 1966; Nagy, 1980; Nagy and Costa, 1980). One assumption
violated in measurement of water fluxes of rapidly growing juveniles is that of constancy of the body water pool. However, the calculations were adjusted accordingly (Dove and Freer, 1979), so growth of the juveniles probably led to little error.

Another assumption violated was that of constancy of fluxes throughout the measurement period, as milk intake can change within a measurement period. Errors in calculated milk consumption caused by changing milk intakes in mule deer (Odocoileus hemionus) fawns were largest during the declining phase of milk intake, but always within 5 % of the true intakes (Carl and Robbins, 1988). Lactation is more prolonged in the koala than in mule deer. Thus milk intake probably changes less rapidly, so errors caused by changing rates of intake might be expected to be less in the koala than in the deer.

The third assumption likely to have been violated is that water should not enter the body through skin or respiratory surfaces. During early lactation, before the young is furred, water vapour is likely to be exchanged between the lungs and skin of the young and the bare skin of the interior of the pouch. In Mus, vapour exchange accounted for half the water intake of the naked young (Baverstock and Green, 1975; Friedman and Bruno, 1976). Dove et al. (1989) modelled the effects of vapour exchange to the calculated milk intake of juvenile tammar wallabies. In small (100g), naked juveniles within the pouch, vapour exchange led to large overestimates in the calculated milk intake. With a large magnitude of vapour exchange the body water pool of the young behaves as a part of the mother's much larger pool (Dove et al., 1989). However, in larger (400g), furred young, vapour exchange leads to smaller errors, and exchange is also likely to be small as the young are furred and spend periods outside the pouch (Dove et al., 1989). In the tammar, vapour exchange is unlikely to cause serious error in the estimation of milk intake after 200 days (450-500g) (Dove et al., 1989). In the koala, the duration of lactation and timing of development are similar to those of the tammar wallaby (Russell, 1982a), so the magnitude of errors due to vapour exchange should have had a similar time course. In this study, milk intake measurements were made only in furred young, thus avoiding serious error due to vapour exchange. Only three measurements of milk intake were made prior to 200 days, all after 175 days. In two of these, milk intake was overestimated by 10-30 %, as the ratio of milk-water intake to total water intake was around 110-130 %. However, this error is unlikely to affect the conclusions of this study as milk intake is still low at that stage.
Recycling of isotopes between mother and young, as could occur with vapour exchange (discussed above) or maternal consumption of juvenile excreta, can also lead to errors in calculated milk intake. In rodents and the dingo, 30 to 50% of the water secreted as milk was recycled to the mother, possibly leading to large errors in the calculated milk intakes (Baverstock and Green, 1975; Baverstock and Elhay, 1979). Dove and Cork (1989) found about 40% recycling of water from a 210-day old tammar young, but this level of recycling led to only 1-2% error in estimates of milk-water intake (Dove et al., 1989). The error was small primarily due to the small size of the body water pool of the tammar juvenile compared to that of its mother. Error from this source would be greater in multiparous species where the water pool of the litter is a greater proportion of the mother's (Dove et al., 1989). The single koala juvenile represents a proportion of the mother's body water pool similar to, or lower than, that in the tammar, and so recycling of water via excreta is unlikely to cause large errors in calculated milk-water intakes. Moreover, koalas do not consume excreta of the young or clean the pouch, and after the initial pouch exit (about 210 days) the young urinate and defaecate outside the pouch (Smith, 1979a, c); therefore errors due to recycling in this way are probably minimal.

Juvenile koalas consume a form of maternal faeces, known as "pap", from the ages of 170 to 210 days, immediately before and around the time of first pouch emergence (Fleay, 1937; Minchin, 1937; Smith, 1979c; V. Thompson, 1987). The exact function of "pap" feeding is unknown, though it has been suggested that it is to inoculate the juvenile gut with the maternal gut flora (Lee and Martin, 1988), act as a vitamin B supplement, train the young to follow maternal leaf choice, and/or activate the detoxification systems of the young (comments in symposium discussion, Bergin, 1978).

Because "pap" is a soft, fluid form of maternal faeces it contains a high proportion of maternal body water. Consumption of water in "pap" is indistinguishable from milk-water intake measured by water fluxes, and so consumption of large amounts of "pap" would lead to overestimation of milk intake. The magnitude of "pap" consumed is unknown; Smith (1979c) observed it only four times, Fleay (1937) 10 times, and Minchin (1937) for about an hour every second or third day for several weeks. None of these authors made observations at night or could quantify "pap" consumption, so the error in milk intake due to "pap" consumption is unknown. However, in the present study most measurements of milk intake were made after the reported period of "pap" feeding; values later than about 240 days should not be affected, and only the earliest milk intake measurements are subject to this error. Therefore, estimates of peak milk production are unlikely to have been affected by "pap" consumption.
The double isotope method also requires the specific activity of $^3$H$_2$O tracer to be the same in milk-water as in maternal body water (Holleman et al., 1975). This assumption was not tested in this study, but has been found to apply in both tammar wallabies (Dove and Cork, 1989) and ringtail possums (Munks, 1990), so is unlikely to be a source of error.

In summary, the estimates of milk-water intake from around the time of pouch exit and beyond are likely to be accurate to within 2.5-5% of the true milk-water intake, but the earliest measurements may be overestimates due to vapour exchange and "pap" consumption.

By comparison, most other methods used for measuring milk intake, such as timed sampling and weighing methods, can have large errors associated with them (Oftedal, 1984), or cannot be used after the start of weaning, as with $^{22}$Na transfer (Green et al., 1988) and $^3$H$_2$O dilution (Dove and Freer, 1979). The advantage of the $^{22}$Na method is that it is not affected by vapour transfer and so can be used earlier in the pouch life of marsupials (Green et al., 1988; Dove and Cork, 1989).

### 5.4.2 Milk production by koalas

The peak milk-energy output of the koala was the lowest recorded for any mammal of equivalent mass (Table 5.4), only 18-28% of the predicted peak milk-energy output for eutherians. The other two herbivorous marsupials for which milk production has been measured, the tammar wallaby and common ringtail possum, also have low peak milk-energy output, but 30-110% greater than in the koala (Table 5.4). On the basis of litter metabolic mass (Oftedal, 1984), the milk-energy output of marsupials is also low, and that of the koala is the lowest, but the difference is less than that per maternal metabolic mass (Table 5.4). This suggests that koala young are relatively small at peak lactation, i.e. weaning commences relatively early.

Milk-energy output at peak lactation is proportional to metabolic body mass ($kg^{0.75}$) (Payne and Wheeler, 1967; Linzell, 1972; Hanwell and Peaker, 1977; Oftedal, 1984), while the duration of reproduction is proportional to physiological time ($kg^{0.25}$) (Sacher and Staffeldt, 1974; Blueweiss et al., 1978; Zeveloff and Boyce, 1986; Taylor and Murray, 1987). Cork and Dove (1989) argued that the total energetic requirements for reproduction should be proportional to mass, as the product of output ($kJ.kg^{-0.75}.d^{-1}$) and duration ($d.kg^{-0.25}$).
Table 5.4 Milk-energy output in non-dairy species (adapted from Oftedal, 1984).

<table>
<thead>
<tr>
<th>Species</th>
<th>Mass (kg)</th>
<th>Milk-energy output (kJ.kg$^{-0.75}$ d$^{-1}$)</th>
<th>Total ME (MJ.kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(kJ.kg$^{-0.83}$ d$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>Marsupials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koala</td>
<td>6</td>
<td>99-122</td>
<td>421-642</td>
</tr>
<tr>
<td>Ringtail possum</td>
<td>1</td>
<td>154</td>
<td>519</td>
</tr>
<tr>
<td>Tammar wallaby</td>
<td>4.7</td>
<td>207</td>
<td>568</td>
</tr>
<tr>
<td>Eutherians</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>57</td>
<td>146</td>
<td>715</td>
</tr>
<tr>
<td>Baboon</td>
<td>16.7</td>
<td>162</td>
<td>845</td>
</tr>
<tr>
<td>Dorcas gazelle</td>
<td>20.6</td>
<td>371</td>
<td>883</td>
</tr>
<tr>
<td>Ibex</td>
<td>30.3</td>
<td>336</td>
<td>891</td>
</tr>
<tr>
<td>Black-tailed deer</td>
<td>49.8</td>
<td>494</td>
<td>979</td>
</tr>
<tr>
<td>Sheep</td>
<td>52.6</td>
<td>586</td>
<td>1054</td>
</tr>
<tr>
<td>Red deer</td>
<td>85.3</td>
<td>320</td>
<td>946</td>
</tr>
<tr>
<td>Reindeer</td>
<td>107</td>
<td>332</td>
<td>1188</td>
</tr>
<tr>
<td>Elk</td>
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<td>301</td>
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<tr>
<td>Cattle (beef)</td>
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<td>278</td>
<td>816</td>
</tr>
<tr>
<td>Horse</td>
<td>515</td>
<td>344</td>
<td>879</td>
</tr>
<tr>
<td>Brown rat</td>
<td>0.2</td>
<td>1008</td>
<td>891</td>
</tr>
<tr>
<td>Mink</td>
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<td>602</td>
<td>887</td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>0.98</td>
<td>354</td>
<td>640</td>
</tr>
<tr>
<td>Skunk</td>
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<td>686</td>
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<tr>
<td>Black bear</td>
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<td>990</td>
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<tr>
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<td>Northern elephant seal</td>
<td>509</td>
<td>900</td>
<td></td>
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<tr>
<td>Weddell seal</td>
<td>447</td>
<td>757</td>
<td></td>
</tr>
<tr>
<td>Northern fur seal</td>
<td>38</td>
<td>590</td>
<td></td>
</tr>
<tr>
<td>California sea lion</td>
<td>88</td>
<td>356</td>
<td></td>
</tr>
</tbody>
</table>

*a The total metabolic energy requirements for all reproduction. This was calculated from the sum of the total lactational output and the ME requirements of gestation (Oftedal, 1985), assuming 65% efficiency of conversion of ME to milk-energy.

b From Oftedal (1985)

c Litter metabolic mass is (Oftedal, 1984)

References:
1. This study
2. Munks (1990)
4. Coward et al. (1979)
5. Buss and Voss (1971)
7. Sadleir (1980)
10. McEwan and Whitehead (1971)
12. Yates et al. (1971)
13. Oftedal et al. (1983)
15. Cowie (1969)
16. Partridge et al. (1986)
17. Gittleman and Oftedal (1987)
18. Oftedal, Iverson and Boness (1987)
19. Costa et al. (1986)
20. Tedman and Green (1987)
22. ARC (1980)
23. Oftedal, Boness and Tedman (1987)
The peak milk-energy yield of the koala was low, but the total metabolizable energy requirement for reproduction in the koala (19-29 MJ.kg\(^{-1}\)) was within the range for other mammals (Table 5.4). This was because the duration of reproduction (approx 400 days, or 270 d.kg\(^{-0.25}\)) was longer than in eutherians (S. Thompson, 1987), or other marsupials (Russell, 1982a), of similar mass. Cork and Dove (1989) interpreted a similar pattern in the tammar wallaby as spreading the load of reproduction over a long period, thus reducing the magnitude of daily requirements, especially at peak, but allowing a similar total input to reproduction as other mammals. They suggested that this was an adaptation to physiological constraints on ingestion and digestion in this small grazer.

It seems that spreading the load of reproduction is a strategy common to the three marsupials for which data on milk-energy output are available. All are small herbivores, and the two with lowest peak output, the koala and ringtail possum, are arboreal folivores, with the associated digestive constraints and energetically conservative characteristics. In the koala, spreading the load of reproductive requirements with the concomitant low peak-requirement is another energetically conservative characteristic, along with its low metabolic rate (This study, Chapter 6; Degabriele and Dawson, 1979; Nagy and Martin, 1985) and low level of activity (Robbins and Russell, 1978; Nagy and Martin, 1985). One of the major ways that koalas meet the energy requirements of reproduction on a limited energy budget is by spreading the load of reproduction, thus reducing the daily demands. Reduction in peak demand is especially important as koalas do not use maternal energy stores to supplement the input of dietary energy to reproduction (Chapter 6) and the physiological limits to food intake by a herbivore of this size combined with the low availability of nutrients in *Eucalyptus* foliage limit the extra energy available for reproduction.

The considerable difference in peak milk output and total energy requirements of reproduction between the two years of the study indicate flexibility in the strategy of energy allocation by koalas. In 1991, when rainfall was low and new foliage growth sparse (Chapter 8), energy allocation to reproduction (both peak and total) was about 65 % of that in 1990, when availability of new foliage was much greater. The major difference in the pattern of reproductive output by female koalas between the years was the lipid content of the milk (Chapter 5) and consequently the lipid intake of the young. This could indicate maternal control of reproductive output; when nutrient availability was relatively high more energy was diverted to the young than when it was low. Such increases in diet quality, especially lipid and protein, are associated
with increases in yield of milk-lipid in other species (Clarke, 1975; Prentice, 1980; Thomas and Martin, 1988).

5.4.3 Requirements of juvenile koalas

The growth rate of koalas is low; the Gompertz growth constants are 0.0049-0.0053 (Martin and Handasyde, 1990), about half that predicted for a marsupial of their asymptotic mass (Lee and Cockburn, 1985). Juvenile koalas are also lean (78 % body water) compared with ringtail possum juveniles (65 %, Munks, 1990) and tammar juveniles (75.3 %, Dove and Cork, 1989), and therefore probably do not have sizeable energy stores.

At permanent pouch exit in 1991, juvenile koalas (619 g) consumed 334 kJ.d⁻¹ of milk-energy (314 kJ.d⁻¹ metabolizable energy; 94 % digestible (Walker and Vickery, 1989) and almost totally metabolizable), and 1.8 g of leaf dry matter (17 kJ.d⁻¹, calculated as in Section 6.2.1.5). The total intake was 331 kJ.d⁻¹ metabolizable energy. The FMR at that stage was 302 kJ.d⁻¹ (from Equation 5.2), leaving only 29 kJ.d⁻¹ (9 % of the metabolizable energy intake) for growth. Similarly, at peak lactation, just after pouch exit, there was only 7 kJ.d⁻¹ available for growth. This indicates that the period around pouch emergence, when the young has the greatest requirements met by milk alone, has the potential to be a period of nutritional stress for the young. Energy requirements rise as the young becomes thermally competent (Wallis and Maynes, 1973; Loh and Shield, 1977) and leave the pouch. In tammar juveniles, liver glycogen levels fall by 60-75 % in the weeks following pouch exit, and this is a period of high mortality compared with other stages of development (Janssens and Rogers, 1989). Juvenile mortality in this study was also greatest at, or just before, pouch exit (unpublished personal observations).

After pouch exit, foliage intake assumes increasing importance to the energy intake of the young, and yet weaning is not complete for nearly five more months. During the weaning period, protein levels of the milk increase (Figure 4.5) and the milk-protein intake of the young decreases more slowly than intake of the other components (Figure 5.10). The protein and lipid intake of the young from milk during the later part of weaning may act as supplements to foliage intake. With the low maternal-energy-transfer of koalas, foliage must rapidly form the bulk of the energy intake of the young. If "pap" feeding inoculates the young with appropriate micro-organisms for digestion of foliage, or prepares the detoxification systems of the young, it may be very important in assuring that the transition to a foliage diet is rapid.
Output of milk-energy varied between the two years studied. In 1990 juveniles consumed 209 kJ.d\(^{-1}\) more milk-energy at pouch exit than in the same period in 1991. This meant that there was 204 kJ.d\(^{-1}\) metabolizable energy available for growth, seven times as much as at pouch exit in 1991 (29 kJ.d\(^{-1}\)). Both peak and total lactational energy output were 35% lower in 1991 than 1990. Surprisingly, the leaf intake by juveniles was no greater in 1991 than in 1990, and began no earlier; the contrary might be expected, to compensate for the low milk production in 1991. This may indicate constraints on foliage intake; all the juveniles may have been consuming the maximum amount of foliage they were able to, thus limiting their ability to compensate for maternal shortfall in energy transfer.

Despite lower maternal allocation of energy to the young in 1991 than 1990, and apparent inability of the juveniles to compensate by increasing foliage consumption, growth of the young was similar in the two years (Table 5.3). Age of juveniles was determined from head size early in lactation (100-150 days, approx 100 g), before the bulk of growth, and later ages calculated from the earlier one. If early growth was markedly faster in 1990 than 1991 then this procedure would have overestimated age of 1990 juveniles and underestimated ages of 1991 juveniles, decreasing the chance of detecting mass differences at some calculated age. However, as age was determined early, error of this sort was probably minimized.

Laboratory rats deprived of food for 12 days reduced their energy requirements to only 40% of their normal requirements (Westerterp, 1977). This was achieved partly by reduction in activity, but also by lowering basal metabolic rate by 35% below the normal basal metabolic rate, partly as a consequence of lowered core temperature and increased insulation (Westerterp, 1977). If the juvenile koalas in 1991 responded to low milk production by similarly decreasing FMR (i.e. if FMR was higher in 1990 than 1991), then the difference in energy available for growth of the young between the years may have been less than estimated, explaining the lack of difference in growth of young between years. Alternatively, the intake of protein rather than energy per se may limit growth in juvenile koalas, as in milk-fed lambs (Black et al., 1973; Black and Griffiths, 1975). Lambs fed a diet with 12% protein required 37% more gross energy intake per unit of body weight gain than lambs fed 29% protein (Walker and Norton, 1971). The greater milk-energy intake of juvenile koalas in 1990 was largely due to greater lipid intake; protein intake was not much greater than in 1991. Young koalas were lean, thus they did not store excess lipid, so mass gain may have been limited by the rate of muscle deposition, in turn limited by protein intake, which was similar in the two years.
Female koalas face a tradeoff in the allocation of energy to reproduction. Digestive constraints limit their acquisition of energy and therefore the energy available for reproduction; thus koalas have an extremely low rate of transfer of energy from mother to young. However, requirements of the young and limitations to their ability to compensate for shortfalls in maternal energy transfer set the lower limit for the rate of maternal allocation of energy to reproduction. In order to reproduce successfully, female koalas must raise their young at least to the minimum size for complete folivory in koalas (Millar, 1977). Because they are seasonal breeders (Martin and Handasyde, 1990), there is also a time limit on lactation if the female is to breed at all in the next year. Such a time limit may limit their potential to minimize daily and peak energy requirements of lactation by further extending lactation, as is done under poor nutritional conditions by some primates (Lee et al., 1991), seals (Trillmich, 1986) and elephants (Lee and Moss, 1986). Therefore there is some rate of maternal expenditure below which the production of viable young is not possible. The milk-energy output of female koalas in 1991 in this study must lie close to that level. The higher level of maternal input to the young in the 1990 season probably reflects a greater availability of nutrients to the mother in that year, and therefore a greater ability to transfer energy to the young.

The narrow gap between maternal ability and juvenile requirements in the transfer of energy by koalas emphasises the importance of diet composition, digestive constraints and adaptations, energy conservation and the development of the young to the biology of the koala.
CHAPTER SIX
ENERGETICS DURING LACTATION

6.1 INTRODUCTION

In most mammals, the energetic demands of reproduction are met primarily by increases in food energy intake (Chapter 1). However, increases or decreases in basal metabolic rate (BMR) (Nicoll and Thompson, 1987), use of stored energy (Section 1.4.3) and other changes to the maternal energy budget can contribute substantially to meeting energy requirements for reproduction (Speakman and Racey, 1987; Gittleman and Thompson, 1988).

The energetic demands of reproduction are greatest during the period of peak lactation in both marsupials and eutherians (Table 1.1; Hulbert and Gordon, 1972; Kennedy and Heinsohn, 1974; Smith et al., 1982; Green and Eberhard, 1983; Nagy and Suckling, 1985; Lee and Nagy, unpublished cited in Lee and Cockburn, 1985; Gittleman and Thompson, 1988; Kenagy et al., 1990; Munks, 1990).

Although the energy demands of lactation can be high, because most of the extra energy is exported as milk, increases in metabolic rate are generally relatively low (Section 1.4.2; Trojan and Wojciechowska, 1967; Randolph et al., 1977; Moen, 1978; Studier, 1979; McLure, 1987; Prentice and Whitehead, 1987; Racey and Speakman, 1987; Weiner, 1987a), but some large increases have been reported in species with low BMR (Nicoll and Thompson, 1987). In the golden-mantled ground squirrel, Spermophilus saturatus, with a body mass of 232 g and an average litter size of 2.7, females in late lactation have the highest field metabolic rate (FMR) of any segment of the population and metabolize and export as milk over 30% of their total annual energy requirements in one month (Kenagy et al., 1990). Despite this intensity of reproductive output, the FMR of lactating females at peak lactation is only 17% (not significantly different, P>0.05) greater than that of non-lactating females and the increase with increase of litter size from 1 to 5 is only 10% (Kenagy et al., 1990). Because the increase in FMR due to reproduction in Spermophilus is small compared with the increase in total energy requirement, the energy allocated to reproduction is more likely to be constrained by limits to food intake than by limits to sustainable metabolic rate (Kenagy, 1987; Kenagy et al., 1989b; Kenagy et al., 1990).

In some instances, the increases in metabolic rate during reproduction are less than expected. In these cases there may be compensation for reproductive energy
expenditure by reduction of other components of the maternal energy budget (Section 1.4.2; Randolph et al., 1977; Prentice and Whitehead, 1987; Racey and Speakman, 1987).

The mass specific FMR of lactating female common ringtail possums, *Pseudocheirus peregrinus*, around the period of peak lactation was 25% greater than that of non-lactating females, but not significantly different in the earlier stages of lactation (Munks, 1990). Similarly, the early stages of gestation and lactation in the tammar wallaby, *Macropus eugenii*, place relatively low demands on the energy metabolism of the mother, but rise to a peak at permanent pouch exit (Cork and Dove, 1989). In the koala, the pattern of milk production is similar to that in the tammar (Figure 5.12), and the maximum energy demands of reproduction are expected to occur at peak lactation, around 250-300 days.

The field metabolic rate (FMR) of non-reproductive adult female koalas measured in late winter and early spring on the south-east coast of Victoria was 628 kJ.kg$^{-0.58}$d$^{-1}$ (7.8 kg body mass), and food dry matter intake was 350 g (Nagy and Martin, 1985). Mass specific FMR of the larger male koalas was lower than that of the females.

Chapters 4 and 5 quantified the transfer of energy from mother to young in koalas. This chapter examines the way that the energetic demands of lactation are met in koalas, via a comparison of energetics, and of water and food intake in lactating and non-lactating female koalas.

6.2 MATERIALS AND METHODS

6.2.1 Daily energy expenditure and water flux

Energy expenditure (Field metabolic rate, FMR) and water flux were estimated using the turnover of isotopic body water tracers (Lifson and McClintock, 1966). Water flux in adult females was measured using the dilution and washout of an intravenous dose of tritiated water (HTO). FMR was estimated by the doubly-labelled water method, using the "low enrichment" procedure of Nagy et al. (1990). In this method the production of carbon dioxide by the animal is estimated by the differential rates of turnover of $^{18}$O, labelling both water ($\text{H}_2^{18}\text{O}$) and carbon dioxide ($\text{C}^{18}\text{O}_2$), and $^3\text{H}_2\text{O}$ (tritiated water), which labels body water.

Koalas were captured, handled and measured as described in Chapter 3.
6.2.1.1 Isotope injection and blood sampling

After the koala had been sedated, a 22 gauge intra-venous catheter (Insyte 22G, 2.5 cm IV Placement Unit, Deseret Becton Dickinson) was inserted into the cephalic vein found superficially on the cranio-medial aspect of the forearm. The catheter was then plugged and taped firmly to the forearm with "Leukoflex" (BDF) tape.

Blood samples were taken through the injection plug and collected into glass tubes (Vacutainer SST, Becton Dickinson) very carefully to avoid haemolysis. The volume of sample collected was dependent on the isotope assays required. For FMR estimation 10 ml blood samples were required from the adult females, but for water turnover estimations only 5 ml was collected. The total volume of sample taken at any one capture was no more than 5 % of the estimated total blood volume.

Samples were stored at 5°C for the remainder of the day, then stored at -20°C until analysis. After the discovery in late 1990 that some containers cracked when frozen (also see Nagy et al., 1990), water turnover (i.e. HTO) samples were collected into plastic tubes and sealed with Parafilm before freezing, while the DLW samples were not frozen but kept at 5°C until analysis.

After an initial blood sample was taken to determine background concentration of isotopes, the isotope solution was injected into the vein via the catheter, ensuring that there was no leakage of the solution out of the vein or onto the skin. The dose administered was determined by weighing the syringes to the nearest 0.01 g before and after the dose, on a portable, battery-operated digital balance. Immediately after the isotope injection the catheter was flushed with 2.5 ml sterile saline (0.9 % NaCl) to ensure that all of the isotope dose entered the blood stream and that none remained to contaminate the equilibration blood sample.

Isotope doses for estimation of FMR were 1.6 ml.kg⁻¹(body mass) of sterile saline (0.9 % NaCl) containing approximately 10 atom percent excess ¹⁸O and 4 MBq tritium. When only water turnover was estimated, the isotope dose was 1 ml.kg⁻¹(body mass) of sterile saline (0.9 % NaCl) containing 4 MBq of tritium.

After flushing the catheter with saline, the whole catheter and plug unit was covered with an elastic, self-adhesive bandage (Expandover) and the koala placed in a holding container (Jetbox animal travelling box) for the equilibration period.

After the 2 hour equilibration period (see below) a further blood sample was taken for isotope analysis. The catheter was cleared immediately before the sample by withdrawing and discarding 1 ml of blood. The koala was then released into the tree
from which it was caught. After 6-8 days (see below) the koala was recaptured and its blood resampled.

**6.2.1.2 Equilibration time**

To determine the time necessary for equilibration of isotope tracers, eight adult captive koalas (6 female, 2 male) at Featherdale Wildlife Park (Sydney) were injected with an intravenous dose of 2 ml.kg\(^{-1}\)(body mass) deuterated water (D2O, 99 atom percent excess) in the same way as described above. The koalas were held in small individual holding pens and were able to sit on a tree branch. Blood samples (2 ml) were taken at 60, 90, 120, 150 and 180 min after injection into Li-Heparin treated tubes, sealed, and stored first on ice and then at -20°C until analysis.

On the basis of the results of the equilibration experiment, a period of two hours was selected to allow equilibration of isotope doses in the field. Two hours was also the equilibration time selected by Nagy and Martin (1985) in their study of the FMR of koalas, but without experimental justification.

**6.2.1.3 Time to recapture**

The accuracy of estimation of isotope fluxes is partly determined by the interval until resampling (Nagy, 1983). If little turnover has occurred, or the levels have declined to close to background levels, estimates are particularly sensitive to small errors in isotope analysis (Nagy, 1983). Nagy (1983) considered that reliable CO\(_2\) production estimates are obtained with an interval to recapture of one to two biological half-lives of the \(^{18}\)O label, and that water fluxes are accurate for much longer intervals (five half-lives of the tritium label). These criteria were accepted with the following proviso; because the isotope levels used in this study were much lower than those considered by Nagy (1983), and were therefore closer to background levels, the interval to recapture and sampling was chosen to be closer to one than to two half-lives of the \(^{18}\)O isotope.

The appropriate interval to recapture and blood sampling was determined from Nagy's (1983) relationship between isotope turnover and body size,

\[
T_{1/2} = 0.151 M(g)^{0.444} \quad \text{Equation 6.1}
\]

where \(T_{1/2}\) is the expected half-life of \(^{18}\)O in a mammal of mass \(M(g)\). Nagy and Martin (1985) used an interval of 8 days. The interval to recapture in this study was 6-8 days.
Even though the biological half-life of the tritium label was expected to be longer than that of the $^{18}$O label, the same interval to recapture (6-8 days) was used in the measurements of water turnover only (Table 3.4). This was so that those measurements could be made concurrently with reliable estimates of water turnover in the juveniles, which were expected to have a shorter $T_{1/2}$ (tritium) than the adults.

### 6.2.1.4 Analytical methods

Before isotope analysis, blood samples were vacuum sublimated (Byers, 1979), and the sublimated water was trapped in a liquid nitrogen trap.

Tritium activity was assayed by liquid scintillation spectroscopy. Duplicate aliquots of 100 µl were each pipetted into 10 ml liquid scintillation cocktail (PCS II Amersham) in 20 ml plastic scintillation vials (Packard). The vials were then each counted for 3 minutes in a Packard Tricarb liquid scintillation spectrometer. Activity (counts per minute) was corrected to disintegrations per minute using the quench indication parameter relative to the internal standard of the spectrometer. Because all samples from any one estimation were assayed in one analysis run, and well before considerable decay of the tritium activity (radioactive $T_{1/2} = 12.3$ years), no correction for radioactive decay was necessary.

The analysis of the $^{18}$O isotope was performed by Isotope Ratio Mass Spectroscopy (Nagy et al., 1990) on 2 ml aliquots by Metabolic Solutions Inc., Acton MA.

Dilutions of the appropriate injection solutions to levels similar to those in the body water were made according to mass and analysed with the blood samples so that the precise isotope dose delivered by mass could be calculated.

The analysis of $^2$H$_2$O for the equilibration experiment was done as described in Section 5.2.1.

### 6.2.1.5 FMR Calculations

Water influx, efflux, and CO$_2$ production were calculated by the equations of Nagy (1983). The body water pool (TBW) was calculated from the equilibrium dilution of the tritium dose injected, rather than from dilution of $^{18}$O. This was because the TBW calculated from tritium was available for all measurement periods, while TBW from $^{18}$O was only available for the FMR measurements (Table 3.4). The body water pool at recapture was assumed to be the same proportion of body mass as that measured at the initial capture, and changes in the body water pool were assumed to have occurred linearly.
The ratio of CO$_2$ production to energy released (and oxygen consumed, i.e. Respiratory Quotient or RQ) by metabolic processes varies with the substrates metabolised (Blaxter, 1961). In many studies of FMR a generalised conversion factor is applied, based on the type of food utilized by the animal. Gessaman and Nagy (1988) showed that errors in conversion of CO$_2$ production to a thermal equivalent (joules) in fed mammals are small if an RQ of 0.83 is assumed for all herbivores. However, they suggest that the errors are less if the RQ can be measured either directly or from knowledge of the mix of substrates metabolised. Nagy and Martin (1985) estimated digestibility of *Eucalyptus ovata* foliage by koalas from the ratios of manganese in food and faeces, assuming that the Mn was totally indigestible. They then used the composition and digestibility of *Eucalyptus punctata* foliage by koalas (Cork *et al.*, 1983) to calculate a conversion factor of 21.8 kJ.l$^{-1}$CO$_2$. Munks (1990) used a similar approach, assuming that the metabolizability of nutrients in the wild diet of *Pseudocheirus peregrinus* (*Leptospermum laevigatum*), was the same as the measured digestibility of *Eucalyptus amygdalina* by captive animals, and calculated a conversion ratio of 21.23 kJ.l$^{-1}$CO$_2$.

There are problems involved with assuming that components of *Eucalyptus* foliage are metabolized in proportion to their digestibility. Some of the allelochemicals present in *Eucalyptus* foliage in high quantities (Section 2.1.2.2) are also highly digestible (Cork *et al.*, 1983). Cork *et al.* (1983) found that lipid and phenolics contributed 30 and 33 % respectively of the digestible energy intake of koalas fed *Eucalyptus punctata* foliage, but suggested that much of this digestible intake was not available to the animal, being excreted after detoxification. Foley (1987) reported a similar situation in greater gliders fed *Eucalyptus radiata* foliage; the high terpene content of the *Eucalyptus radiata* foliage was largely digestible but not metabolizable, and was excreted in the urine. Calculation of the substrates available for metabolism based on composition and digestibility would in this case lead to errors associated with an over-estimation of the importance of lipid to metabolism.

Foley *et al.* (1990) used an RQ value close to unity, measured in captive, fed greater gliders (Foley, 1984), to calculate energy use from the CO$_2$ production of free-ranging greater gliders. Their conversion ratio was 21.2 kJ.l$^{-1}$CO$_2$, which reflected a reliance on the metabolism of carbohydrates. This value is close to the other values estimated for marsupial folivores (Nagy and Martin, 1985; Munks, 1990).

It was not possible to empirically determine the contribution of different components of the *Eucalyptus* foliage to the energy metabolism of koalas in this study, so I have assumed that the RQ measured by Foley (1984) in fed greater gliders, and the
conversion factor of 21.2 kJ l⁻¹CO₂ is appropriate for koalas. This assumption is unlikely to lead to large errors in the calculated energy use, as evidenced by the similarity of ratios estimated by other authors (Nagy and Martin, 1985; Munks, 1990). Also, any error associated with this assumption will not affect the comparative values of FMR calculated for lactating and non-lactating koalas, unless the two groups are metabolising markedly different substrates.

### 6.2.1.6 Estimation of Food intake

Food intake of animals in doubly-labelled water measurements of FMR is often estimated by dividing the FMR by the average metabolizable energy content of the food, thus obtaining the mass of food required to remain in zero energy balance (see Nagy, 1987 for review, and Nagy and Montgomery, 1980; Nagy and Martin, 1985; Munks, 1990). However, this approach can only be justified in animals that are non-productive. Growing or reproductive animals must take in more metabolizable energy than they catabolize. In the case of growing animals this energy is deposited as body tissue, and in reproductive animals this energy is passed to the young either in utero or as milk. Feeding rates could in these cases only be calculated if the rate of energy deposition or transfer and the efficiency of these processes were known.

Alternatives to the above technique for the estimation of food intake include direct observation and other isotopic techniques. The turnover of ²²Na has proven useful in estimating food intake (Green and Dunsmore, 1978; Green and Eberhard, 1979; Green and Newgrain, 1979; Green et al., 1984; 1991a; Gales, 1989), but the accuracy of this method is dependent on the similarity of sodium levels in different dietary items. This would require validation for a marsupial folivore.

Since the intake of free water by free-ranging koalas (Degabriele et al., 1978; Nagy and Martin, 1985) and recycling back from the young (Section 5.4.1; Dove et al., 1989) are probably negligible, the turnover of water can be used to calculate food intake if all water intake is assumed to be preformed or metabolic water from foliage, and the water content of the foliage is known. This was the method used to calculate food intake in this study.

Water content of the diet was measured by sampling foliage at each capture of a koala and analysing it as in Section 8.2.1. Water content of foliage consumed by each koala in each measurement period was assumed to be the average of that sampled at the beginning and end of the period. This was probably a sound assumption as the range of water content available (Appendix 1) was narrow, although there was the
possibility of selection of certain high water content leaf age classes during some periods.

The quantity of water produced in energy metabolism is dependent on the substrates utilized. Therefore, for similar reasons to those discussed for FMR calculations, the estimate of metabolic water production of free-living greater gliders (0.030 g H$_2$O.kJ$^{-1}$FMR; Foley et al., 1990) was used to calculate the contribution of metabolic water to the water influx of koalas at times when FMR was measured. During the periods of water flux measurement only (Table 3.4), the metabolic water production as a proportion of water influx was assumed to be the average of all the measured values.

Energy balance was calculated from the intake of metabolisable energy minus the measured FMR. *Eucalyptus* foliage was assumed to contain 22 kJ (gross energy).g(DM)$^{-1}$ (Cork, 1984) with a 45 % efficiency of conversion of that gross energy to metabolisable energy by koalas (Nagy and Martin, 1985). The energy balance calculated here does not represent a true energy balance, as it does not account for the energy exported in milk by lactating females, but rather the balance between intake and FMR.

### 6.2.2 Resting Metabolic Rate

Resting metabolic rate (RMR) was measured using flow-through respirometry in five lactating and six non-lactating female koalas at Featherdale Wildlife Park, Sydney in September 1992. RMR was also measured in three dependent juveniles (Chapter 5). Measurements were performed during one period only, timed to coincide with peak lactation in the captive animals.

RMR measurements were made in an air-conditioned room at 20-22°C. Temperatures within the metabolic chamber, measured with a thermocouple permanently inserted in the chamber, ranged from 19 to 24°C. This is well within the reported thermal neutral zone for koalas (Degabriele and Dawson, 1979).

Female koalas were removed from their normal enclosure at the park at 09:00 hr on the day of their RMR measurement and held in individual cages in the temperature controlled room until their measurement. This is their normal resting period as koalas are generally crepuscular or slightly more nocturnal than diurnal (Mitchell, 1990b). Up to their removal from their normal enclosures they had full access to their normal diet, and thus were considered to be fully fed.
The young of lactating females were removed from the mother and left with the other lactating females in the enclosure. At their stage of development, riding on the mother's back, this was not an abnormal situation for captive koalas. Young have been observed to move from adult to adult (John Stark pers. comm.).

Koalas were placed into the metabolism chamber 30 minutes before a measurement was started, in order to acclimate them and give them time to settle. Measurement periods were 180 min. Immediately after the measurement, deep rectal temperature of the koala was measured, and the koala was weighed, measured and released back into its normal enclosure. Observations were made of activity during the measurement when possible, although all efforts were made to disturb the koalas as little as possible.

Efforts were also made to measure the fasted resting metabolic rate of the female koalas at peak lactation. Due to the very long digesta retention time in koalas (100-200 hours mean retention time for solutes, Chapter 7; Cork and Warner, 1983), it was not possible to obtain post-absorptive measurements without endangering the health of the study animals. Foley (1984) found that it was not possible to fast greater gliders longer than 24 hours without endangering their health, and used a fasting period of 24 hours to obtain fasted measures. For these reasons the fasting time was standardized to 24 hours before the measurement. Fasted resting metabolic rates were obtained in three lactating and five non-lactating female koalas.

6.2.2.1 Apparatus

Resting metabolic rates were determined by measuring oxygen consumption using negative pressure flow-through respirometry. Room air was drawn through the system 4.0 l.min\(^{-1}\) by a vacuum pump outside the experimental room. Incurrent air was dried with self-indicating Drierite (anhydrous calcium sulphate) before and after passing through the respirometer.

The respirometer was constructed from a 60 litre plastic garbage bin, with ports for entry and exit of air, and a clear perspex lid sealed with silicon vacuum grease. It contained a branch fixed with screws through the side of the chamber at three points. A thermocouple wire was passed through a hole in the side of the chamber to protrude approximately 20-30 mm into the chamber.

The silica gel was contained in 70 mm I.D. acrylic tubing with rubber stoppers and 15 mm I.D air lines passed through holes in the stoppers.
The flow rate through the respirometer was controlled by a Brookes 5871-BZ Thermal Mass flow controller which had previously been calibrated using a Toyo volumetric flow meter. The flow controller was positioned between the respirometer and the vacuum pump (Figure 6.1).

Figure 6.1 The flow-through respirometry apparatus used for the measurement of resting metabolic rate.

The air analysed was subsampled from the main excurrent air between the chamber and the Brookes Flow controller via a smaller diameter air line inserted with a T-connector between the respirometer and the flow controller (Figure 6.1). Air was drawn through this line using an Ametek R-2 Flow controller and pump at
100 ml.min\(^{-1}\). The subsampled air passed through silica gel then through the Datex Carbon dioxide analyser before absorption of the CO\(_2\) by Carbasorb (self-indicating KOH). It then passed through the sensing cell (Ametek N-37M) of the Ametek S-3A/ll electro-chemical Oxygen analyser, before the pump and flow meter. It was necessary to subsample the excurrent air as the Oxygen analyser could not accurately measure at flow rates much above 150 ml.min\(^{-1}\).

The Ametek S-3A/ll Oxygen analyser had two sensing cells. The second cell was used to analyse dried, CO\(_2\)-free room air and the response subtracted from the first. The CO\(_2\) and O\(_2\) meters had been previously calibrated with pure nitrogen and known certified gas mixtures. Each morning the oxygen analyser was adjusted to 20.96% using room air and the output of the two cells equated, and the CO\(_2\) meter zeroed.

Output from both the CO\(_2\) and O\(_2\) analysers was acquired on an IBM compatible computer using Sable Systems data acquisition software and an external analog-to-digital converter.

Temperatures of the room, chamber, incurrent and excurrent air from the Ametek flow controller were monitored half hourly during RMR measurements.

### 6.2.2.2 Calculations

The rate of oxygen consumption (V\(_{O_2}\)) was calculated using equation 6.2 (Withers, 1977),

\[
V_{O_2} = \frac{V_E (F_{I O_2} - FE_{O_2})}{(1 - F_{IO_2})}
\]  

Equation 6.2

where \(V_E\) is the flow rate, \(F_{I O_2}\) is the fractional concentration of oxygen entering the system and \(FE_{O_2}\) the fractional concentration of oxygen leaving the respirometer chamber. The flow rate through the primary air line controlled by the Brookes flow controller was corrected for CO\(_2\) (as the analytical line had CO\(_2\) absorbed) and all the rates were corrected to STPD. The total flow rate through the respirometry chamber was calculated as the sum of the flow through the primary and analytical air lines.

The accuracy of the CO\(_2\) analyser used has since come into doubt. Accordingly the CO\(_2\) production data have been discarded. The CO\(_2\) data was still used in correction of pressures in the O\(_2\) analysis line (see above) as only small error would have resulted. The RE of fed koalas was around 1.5 (as measured) compared with an RE close to 1 for fasted koalas. I have assumed that the higher RE in fed koalas indicates a primary reliance on carbohydrate metabolism, while fasted koalas relied more on
metabolism of fats. The conversion factors used to calculate the energetic equivalent of oxygen consumption reflect that difference. The conversion factors used are 20.9 kJ.l⁻¹ for fed koalas and 19.8 kJ.l⁻¹ in fasted koalas (Gessaman and Nagy, 1988).

### 6.2.3 Statistical Methods

Almost all features of life history and energetics are correlated with body mass in a non-linear way (Peters, 1983; Schmidt-Nielsen, 1984). In order to compare measures of metabolism such as FMR and food and water intake, the effects of differing body size must be considered. In interspecific comparisons in the literature allometric scaling exponents have been empirically derived by fitting the available data to curves of the form:

\[ \ln (Y) = C + B \ln (X), \]

**Equation 6.3**

where \( Y \) is the feature of metabolism of interest, \( X \) is body mass, and \( B \) and \( C \) are constants.

Values of the exponent \( B \) vary between taxonomic and ecological groupings (McNab, 1986; Nagy, 1987). Values of \( B \) for marsupials are 0.576 and 0.67 for FMR and food intake respectively (Nagy, 1987), 0.602 for water intake (Nagy and Peterson, 1988), and 0.737 for standard metabolic rate (Dawson and Hulbert, 1970). However, these exponents may not accurately reflect the intraspecific relationship of metabolism to body mass (Andrews and Pough, 1985). As this study is primarily concerned with the intraspecific comparison of lactating and non-lactating female koalas the above exponents may be inappropriate. There are no empirically derived values of these exponents available for the koala and there is no set of data for adult koalas large or uniform enough in this study to derive the values. The variation in FMR with body mass of juvenile koalas (Equation 5.2) indicated an exponent of one. However, those data included measurements from three seasons, and mass and season covaried. Seasonal variation within individual adult koalas was pronounced (Section 6.3.3), so Equation 5.2 may not be applicable to scaling in adult koalas. For these reasons the data were presented both untransformed and transformed using the above exponents (as they are the best available). Wherever possible, comparisons were made on loge transformed data using ANCOVA (Wilkinson, 1989) with loge(mass) as the covariate. Thus, results of the statistical analyses were not dependent on the choice of scaling exponent.

Statistical analyses of the FMR and water-intake data sets were complicated by dependence of the data over time. For this reason multiple comparisons of means
have been avoided in the analysis of data collected over the entire 1990-1992 period and comparisons have been limited to individual or few selected time periods using ANCOVA with a repeated measures design. In the few cases where the data violated assumptions of the ANCOVA, such as equality of variances, the non-parametric Wilcoxon-Mann-Whitney two sample test (Wilkinson, 1989) was used to test for statistical significance. The 5% probability level was used to define statistical significance unless otherwise stated.

Percentage data were arcsin(x)\(^{1/2}\) transformed before statistical comparisons. Regression analyses have been performed by the least squares method (Wilkinson, 1989).

6.3 RESULTS

6.3.1 Equilibration experiment

The intravenous dose of deuterated water equilibrated with body water within 60 to 120 min (Table 6.1). By 120 min the venous D\(_2\)O concentration had reached minima in 6 of the 8 koalas (i.e. 75%).

Table 6.1 Time course of isotope equilibration and measured body water (means ± S.D., n=8).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>(^2\text{H}_2\text{O}) % of final concentration</th>
<th>Body Water (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>72.4 ± 2.9</td>
</tr>
<tr>
<td>60.1 ± 1.1</td>
<td>100.8 ± 2.0</td>
<td>72.4 ± 2.9</td>
</tr>
<tr>
<td>89.0 ± 1.5</td>
<td>98.9 ± 2.0</td>
<td>73.7 ± 2.6</td>
</tr>
<tr>
<td>120.4 ± 1.4</td>
<td>99.8 ± 1.4</td>
<td>73.2 ± 1.9</td>
</tr>
<tr>
<td>151.3 ± 2.0</td>
<td>99.6 ± 1.5</td>
<td>73.4 ± 1.1</td>
</tr>
<tr>
<td>180.5 ± 1.6</td>
<td>100</td>
<td>73.1 ± 1.6</td>
</tr>
</tbody>
</table>

The body water content at 120 min of the captive koalas was 73.2 ± 1.9% of mass.

6.3.2 Body size and composition

There were no significant differences between body masses of lactating and non-lactating female koalas in any measurement period (Table 6.2). Mass of individual koalas did not change between August/September 1990 (Peak lactation, 1990) and

Table 6.2 Average body mass and body water of female koalas in each measurement period (means ± S.D.).

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Status</th>
<th>Mass (kg)</th>
<th>Body water (%)</th>
<th>Body Solids (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>June NL</td>
<td>1(2) 5.45 ± 0.07</td>
<td>68.7 ± 1.8</td>
<td>1708 ± 121</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L² 6.56 ± 1.00</td>
<td>68.0 ± 2.7</td>
<td>2089 ± 304</td>
<td></td>
</tr>
<tr>
<td></td>
<td>July NL</td>
<td>5.69 ± 0.05</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L 6.78 ± 1.00</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aug/Sept NL</td>
<td>6.03 ± 0.35</td>
<td>75.5A ± 1.3</td>
<td>1477 ± 130</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L 6.73 ± 0.96</td>
<td>71.9B ± 1.6</td>
<td>1835 ± 323</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sept/Oct NL</td>
<td>5.75 ± 0.28</td>
<td>72.2 ± 1.2</td>
<td>1599 ± 12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L 6.41 ± 1.02</td>
<td>71.5 ± 1.0</td>
<td>1831 ± 315</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nov.⁴ NL</td>
<td>5.61 ± 0.30</td>
<td>72.4 ± 4.1</td>
<td>1557 ± 313</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BY 6.13 ± 0.92</td>
<td>72.1 ± 2.0</td>
<td>1710 ± 301</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1991</td>
<td>January NL</td>
<td>5.83 ± 1.09</td>
<td>71.6 ± 1.8</td>
<td>1661 ± 358</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L 6.03 ± 0.38</td>
<td>71.0 ± 0.8</td>
<td>1751 ± 136</td>
<td></td>
</tr>
<tr>
<td></td>
<td>May NL</td>
<td>6.25 ± 1.20</td>
<td>76.8A ± 6.0</td>
<td>1488 ± 566</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L 6.32 ± 0.43</td>
<td>71.5B ± 1.7</td>
<td>1802 ± 154</td>
<td></td>
</tr>
<tr>
<td></td>
<td>July NL</td>
<td>6.05 ± 0.41</td>
<td>73.2 ± 3.5</td>
<td>1614 ± 192</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L 6.25 ± 0.47</td>
<td>70.8 ± 1.2</td>
<td>1826 ± 161</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aug/Sept NL</td>
<td>6.02 ± 0.38</td>
<td>75.2 ± 4.4</td>
<td>1499 ± 320</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L 5.89 ± 0.67</td>
<td>73.2 ± 2.8</td>
<td>1569 ± 166</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sept/Oct NL</td>
<td>6.05 ± 0.33</td>
<td>71.4 ± 1.4</td>
<td>1734 ± 126</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L 5.87 ± 0.63</td>
<td>72.8 ± 1.1</td>
<td>1596 ± 195</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oct/Nov. NL</td>
<td>6.03 ± 0.33</td>
<td>71.0B ± 0.3</td>
<td>1749 ± 115</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L 5.98 ± 0.73</td>
<td>72.9A ± 0.9</td>
<td>1624 ± 222</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dec.³ PY</td>
<td>6.17 ± 0.36</td>
<td>72.9B ± 1.7</td>
<td>1666 ± 65</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>BY 5.94 ± 0.58</td>
<td>76.0A ± 2.4</td>
<td>1423 ± 179</td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td>Jan. NL</td>
<td>5.58</td>
<td>71.6</td>
<td>1586</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>BY 5.83</td>
<td>69.1</td>
<td>1802</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PY 5.92 ± 0.59</td>
<td>71.1 ± 1.9</td>
<td>1717 ± 242</td>
<td></td>
</tr>
</tbody>
</table>

Within columns, means with different subscripts differ at P=0.05.
n.d. indicates that these values were not determined due to a balance malfunction.

1 NL Non-lactating females.
2 L Lactating females. These include those females with pouch young or the more advanced back young, but are presented in homogenous groups within each time period.
3 There were no non-lactating females at this time. The females in the PY (pouch young) group were from the NL group but had very small young in the pouch.
4 One female in this period was lactating, but as the young was very small could not be grouped with the other lactating females, as in ³ above.
At the peak of lactation in 1990 (August/September, Chapter 5), non-lactating females gained 0.23 % of their body mass per day, greater than the 0.09 % loss of mass by the lactating females (P<0.05). This may represent a short term difference in energy balance between the two groups, but is not biologically significant over the longer period as long term mass and mass changes did not differ between lactating and non-lactating females (Table 6.2).

Average body water content ranged from 68.0 % to 76.8 %, similar to those recorded in captive animals (Section 6.3.1). The water content calculated from the dilution of \(^{18}\)O was 5.9 ± 4.4 % greater (paired t-test t=-12.241, P<0.001) than that calculated from \(^3\)H dilution.

Body water content of lactating and non-lactating females were different in four periods- August/September 1990, May 1991, October/November 1991 and December 1991 (Table 6.2), although the differences were not systematic. In the first two of these periods the body water percentage of lactating females was less than that of non-lactating females, with a reversal of the situation in the later two periods.

The total body solids of the koalas was considered to be a better indicator of tissue mass and possible changes over time than percentage water content, as it represents a composite of the total mass and the water content. The total body solids of lactating and non-lactating koalas did not differ (Table 6.2). Neither was there any significant pattern of change in body solids over time (A/S 90 to Jan 91, mean difference = -34 ± 241, P=0.687; Jan 91 to A/S 91 L, P=0.158; Jan 91 to A/S 91 NL, P=0.36).

Values are not given for the body solids or water in July 1990 as the isotope dose could not be accurately determined due to malfunction of the balance used to weigh the injection syringes. For the calculation of water flux in this period body water was estimated by averaging values obtained for each individual from June and August/September 1990.

In summary, lactating females were not found to differ from non-lactating females in body mass, composition or patterns of mass or composition change (Table 6.2).

6.3.3 Field metabolic rates

Field metabolic rates (Table 6.3) of lactating and non-lactating female koalas differed only in January 1991. Lactating females, with only tiny pouch young at this stage, had an FMR of 1627 ± 157 kJ.d\(^{-1}\) or 579 ± 56 kJ.kg\(^{-0.576}\).d\(^{-1}\), which was significantly greater than that of non-lactating females, 1256 ± 279 kJ.d\(^{-1}\) or
Unfortunately there was only one estimate of FMR available for a non-lactating female in January 1992, so it was impossible to determine if this difference was consistent between years.

At the peak of lactation (August/September 1990), when energetic demands on the mother were potentially greatest, FMR was not significantly different from the non-lactating level (ANCOVA, P=0.432, ln(mass) as covariate P=0.695) (Table 6.3).

### Table 6.3 Field metabolic rate of female koalas during each measurement period (means ± S.D.)

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Status</th>
<th>CO₂ Prodn (ml.g⁻¹.h⁻¹)</th>
<th>FMR (kJ.d⁻¹)</th>
<th>FMR (kJ.kg⁻⁰.⁵⁷₆.d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>June</td>
<td>NL (2)</td>
<td>0.62 ± 0.03</td>
<td>1727 ± 64</td>
<td>650 ± 29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (7)</td>
<td>0.48 ± 0.10</td>
<td>1595 ± 246</td>
<td>543 ± 92</td>
</tr>
<tr>
<td></td>
<td>Aug/Sept</td>
<td>NL (3)</td>
<td>0.57 ± 0.04</td>
<td>1748 ± 183</td>
<td>621 ± 50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (6)</td>
<td>0.55 ± 0.08</td>
<td>1855 ± 113</td>
<td>624 ± 61</td>
</tr>
<tr>
<td></td>
<td>Nov.</td>
<td>NL (2)</td>
<td>0.46 ± 0.16</td>
<td>1307 ± 388</td>
<td>486 ± 159</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PY (1)</td>
<td>0.30</td>
<td>1395</td>
<td>515</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BY (9)</td>
<td>0.41 ± 0.09</td>
<td>1246 ± 228</td>
<td>442 ± 87</td>
</tr>
<tr>
<td>1991</td>
<td>Jan.</td>
<td>NL (7)</td>
<td>0.43B ± 0.08</td>
<td>1256B ± 279</td>
<td>456B ± 83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (6)</td>
<td>0.53A ± 0.06</td>
<td>1627A ± 157</td>
<td>579A ± 56</td>
</tr>
<tr>
<td>May</td>
<td>NL (5)</td>
<td>0.71 ± 0.14</td>
<td>2211 ± 297</td>
<td>777 ± 114</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L (7)</td>
<td>0.65 ± 0.07</td>
<td>2088 ± 348</td>
<td>720 ± 97</td>
<td></td>
</tr>
<tr>
<td>July</td>
<td>NL (5)</td>
<td>0.43 ± 0.05</td>
<td>1337 ± 184</td>
<td>474 ± 55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L (7)</td>
<td>0.41 ± 0.05</td>
<td>1319 ± 215</td>
<td>458 ± 62</td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td>Jan.</td>
<td>NL (1)</td>
<td>0.58</td>
<td>1649</td>
<td>613</td>
</tr>
<tr>
<td></td>
<td>BY (1)</td>
<td>0.49</td>
<td>1457</td>
<td>528</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PY (8)</td>
<td>0.49 ± 0.25</td>
<td>1419 ± 652</td>
<td>518 ± 254</td>
<td></td>
</tr>
</tbody>
</table>

1 The average body mass of each group at each time and the abbreviations of status are as in Table 6.2.

Within a column, means bearing different subscripts differ significantly (P<0.05).

There was however, a pronounced seasonal trend in FMR in both groups of females (Table 6.3). The early winter FMR's (June 1990) were 30% higher than in early summer (November 1990) (difference = 359 ± 181 kJ.d⁻¹ or 117 ± 69 kJ.kg⁻⁰.⁵⁷₆.d⁻¹,

456 ± 83 kJ.kg⁻⁰.⁵₇₆.d⁻¹ (ANCOVA, P=0.019, ln(mass) as covariate P=0.103).
Changes in FMR from summer to winter in 1991 were considered in non-lactating females only, due to the difference in FMR between the groups in summer. In winter 1991 (May) non-lactating females had FMR 70% greater than in summer (January) (difference = 972 ± 187 kJ.d⁻¹, or 343 ± 100 kJ.kg⁻⁰·⁵₇₆·¹. Paired t-test, P=0.002). If the difference between the summer and winter FMR's was due mainly to thermoregulatory requirements, then in June 1990 and May 1991 female koalas expended 22 and 42% respectively of their field metabolic rates in thermoregulation.

In summary, lactating females did not exhibit an elevated FMR at the peak of lactational energy demand. However, they did have a relative increase in FMR early in lactation when the demands of the young were low (Chapter 5). There was also a pronounced seasonal effect on FMR, with much higher energy use during winter than summer.

### 6.3.4 Water and food intake

Average water intake by female koalas varied with reproductive status, season and year, ranging from 221 to 384 g.d⁻¹ (Table 6.4). Water intake was higher in summer than winter and higher in 1990 than 1991. Comparison of the lactating females only (due to the significant differences between the groups) in August/September 1990 and August/September 1991 shows a lower water intake during 1991 (t-test, P= 0.035). This reflects the difference in water content of foliage browsed by the koalas in 1991; 8.6% lower than in 1990 (t-test, P= 0.042). Presumably the lower water content of foliage in late winter and early spring 1991 than the same period in 1990 is related to the low rainfall in 1991. For the period August to November 1990 nearly twice as much precipitation was recorded (365 mm) than the same period in 1991 (184 mm) at the nearby "Goldsmith's" weather station (unpublished Forestry Commission data).

Lactating females had a greater water intake than non-lactating females (Table 6.4). During 1990 this trend was statistically significant only late in lactation, during November (ANCOVA, P= 0.013, ln(mass) as covariate P= 0.002). In 1991 the water intake of lactating females was greater than that of non-lactating females from September/October to December (repeated measures ANCOVA, P= 0.007, ln(mass) as covariate P= 0.055).

Food intake estimates were not made for the June and July 1990 measurement periods because accurate estimates of foliar water content were not available.
<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Status (N)</th>
<th>Water Intake (g.d$^{-1}$)</th>
<th>Water Intake (g.kg$^{-0.602}$.d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>June</td>
<td>NL (2)</td>
<td>328 ± 12</td>
<td>118 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (7)</td>
<td>351 ± 80</td>
<td>112 ± 16</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>NL (2)</td>
<td>306 ± 52</td>
<td>108 ± 18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (7)</td>
<td>384 ± 96</td>
<td>121 ± 22</td>
</tr>
<tr>
<td></td>
<td>Aug/Sept</td>
<td>NL (3)</td>
<td>304 ± 32</td>
<td>103 ± 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (7)</td>
<td>384 ± 85</td>
<td>121 ± 15</td>
</tr>
<tr>
<td></td>
<td>Sept/Oct</td>
<td>NL (2)</td>
<td>275 ± 49</td>
<td>96 ± 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (7)</td>
<td>335 ± 58</td>
<td>109 ± 10</td>
</tr>
<tr>
<td></td>
<td>Nov</td>
<td>NL (2)</td>
<td>249B± 15</td>
<td>88B ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PY (1)</td>
<td>315</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BY (9)</td>
<td>341A± 52</td>
<td>114A± 10</td>
</tr>
<tr>
<td>1991</td>
<td>Jan.</td>
<td>NL (7)</td>
<td>307 ± 78</td>
<td>106 ± 16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (6)</td>
<td>338 ± 26</td>
<td>115 ± 7</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>NL (5)</td>
<td>309 ± 61</td>
<td>102 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (7)</td>
<td>315 ± 53</td>
<td>103 ± 15</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>NL (5)</td>
<td>280 ± 22</td>
<td>95 ± 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (7)</td>
<td>314 ± 34</td>
<td>104 ± 11</td>
</tr>
<tr>
<td></td>
<td>Aug/Sept</td>
<td>NL (7)</td>
<td>246 ± 45</td>
<td>83 ± 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (6)</td>
<td>266 ± 26</td>
<td>92 ± 6</td>
</tr>
<tr>
<td></td>
<td>Sept/Oct</td>
<td>NL (6)</td>
<td>221B± 11</td>
<td>75B ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (6)</td>
<td>261A± 18</td>
<td>90A ± 4</td>
</tr>
<tr>
<td></td>
<td>Oct/Nov.</td>
<td>NL (5)</td>
<td>240B± 26</td>
<td>81B ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (6)</td>
<td>286A± 32</td>
<td>97A ± 6</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>PY (5)</td>
<td>280B± 19</td>
<td>93B ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BY (6)</td>
<td>317A± 32</td>
<td>109A± 8</td>
</tr>
<tr>
<td>1992</td>
<td>Jan.</td>
<td>NL (1)</td>
<td>279</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BY (1)</td>
<td>275</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PY (8)</td>
<td>308 ± 38</td>
<td>106 ± 13</td>
</tr>
</tbody>
</table>

1 The average body mass of each group at each time and the abbreviations of status are as in Table 6.2.
Within a column, means bearing different superscripts differ significantly (P< 0.05).
Table 6.5 Metabolic water production, food water content and food intake (means ± S.D.).

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Status (N)</th>
<th>Metabolic Water (g.d⁻¹)</th>
<th>Food Water Content (%)</th>
<th>Food Intake (g.kg⁻⁰·⁶⁷.d⁻¹)</th>
<th>Energy Balance (kJ.d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>June</td>
<td>NL (2)</td>
<td>52 ± 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (7)</td>
<td>48 ± 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1990</td>
<td>Aug/Sept</td>
<td>NL (3)</td>
<td>53 ± 6</td>
<td>41 ± 3</td>
<td>180 ± 44</td>
<td>55 ± 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (7)</td>
<td>56 ± 3</td>
<td>46 ± 5</td>
<td>250 ± 141</td>
<td>70 ± 37</td>
</tr>
<tr>
<td></td>
<td>Sept/Oct</td>
<td>NL (2)</td>
<td>45 ± 6</td>
<td>188 ± 10</td>
<td>58 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (7)</td>
<td>45 ± 4</td>
<td>231 ± 39</td>
<td>67 ± 11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nov.</td>
<td>NL (2)</td>
<td>39 ± 12</td>
<td>37 ± 1</td>
<td>123⁸⁺ ± 12</td>
<td>39³⁺ ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PY (1)</td>
<td>42</td>
<td>41</td>
<td>240</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BY (9)</td>
<td>37 ± 7</td>
<td>40 ± 2</td>
<td>207³⁺ ± 52</td>
<td>61³⁺ ± 9</td>
</tr>
<tr>
<td>1991</td>
<td>Jan.</td>
<td>NL (7)</td>
<td>38³⁺ ± 8</td>
<td>42 ± 3</td>
<td>194 ± 65</td>
<td>59 ± 16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (6)</td>
<td>49³⁺ ± 5</td>
<td>40 ± 2</td>
<td>192 ± 25</td>
<td>58 ± 5</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>NL (5)</td>
<td>66 ± 9</td>
<td>41 ± 4</td>
<td>170 ± 51</td>
<td>50 ± 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (7)</td>
<td>63 ± 11</td>
<td>44 ± 6</td>
<td>207 ± 77</td>
<td>60 ± 21</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>NL (5)</td>
<td>40 ± 6</td>
<td>47 ± 5</td>
<td>213 ± 31</td>
<td>64 ± 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (7)</td>
<td>40 ± 6</td>
<td>49 ± 2</td>
<td>261 ± 42</td>
<td>76 ± 12</td>
</tr>
<tr>
<td></td>
<td>Aug/Sept</td>
<td>NL (7)</td>
<td>52 ± 2</td>
<td></td>
<td>220³⁺ ± 27</td>
<td>66³⁺ ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (6)</td>
<td>54 ± 3</td>
<td></td>
<td>270³⁺ ± 39</td>
<td>82³⁺ ± 11</td>
</tr>
<tr>
<td></td>
<td>Sept/Oct</td>
<td>NL (6)</td>
<td>51 ± 3</td>
<td>196³⁺ ± 27</td>
<td>59³⁺ ± 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (6)</td>
<td>52 ± 2</td>
<td>243³⁺ ± 28</td>
<td>74³⁺ ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oct/Nov.</td>
<td>NL (5)</td>
<td>51 ± 3</td>
<td>210³⁺ ± 38</td>
<td>63³⁺ ± 12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L (6)</td>
<td>54 ± 4</td>
<td>287³⁺ ± 60</td>
<td>87³⁺ ± 15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>PY (5)</td>
<td>44 ± 4</td>
<td></td>
<td>188³⁺ ± 27</td>
<td>56³⁺ ± 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BY (6)</td>
<td>49 ± 4</td>
<td></td>
<td>255³⁺ ± 42</td>
<td>77³⁺ ± 11</td>
</tr>
<tr>
<td>1992</td>
<td>Jan.</td>
<td>NL (1)</td>
<td>50</td>
<td>42</td>
<td>167</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BY (1)</td>
<td>44</td>
<td>40</td>
<td>156</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PY (8)</td>
<td>43 ± 20</td>
<td>43 ± 5</td>
<td>207 ± 71</td>
<td>63 ± 19</td>
</tr>
</tbody>
</table>

¹ The average body mass of each group at each time and the abbreviations of status are as in Table 6.2.
Within a column, means bearing different superscripts differ significantly (P< 0.05).
The intake of food dry matter did not differ between 1990 and 1991 (Table 6.5) (Aug/Sept 90 vs Aug/Sept 91, t-test, \( P = 0.996 \)) despite greater water intake in 1990, due to the lower water content of the foliage browsed in 1991 (see above).

Lactaters ate more during late lactation than did non-lactaters (Table 6.5). During 1990 this difference was only significant in November (ANCOVA, \( P = 0.005 \), \( \ln(\text{mass}) \) as covariate \( P = 0.002 \)). However during 1991 the difference was evident from the August/September to the December measurement period (ANCOVA, \( P = 0.001 \), \( \ln(\text{mass}) \) as covariate \( P = 0.022 \)) (Figure 6.2).

The energy balance of lactating females was not significantly different from that of non-lactating females at any time, though the trend was toward a more positive energy balance in lactating females. The trend approached statistical significance (0.1\( < P > 0.05 \)) in November 1990 and July 1991.

Figure 6.2 Intake of foliage by female koalas (means ± SE).

The extra requirements of lactation amounted to 14,170 g, or 4,266 g.kg\(^{-0.67}\), dry mass of foliage, an increase of 20 % in food intake over the year (Table 6.6). The increase in food intake at peak lactation in 1991 (September/October, Chapter 5) was 27 % and the greatest increase in 1991 was 37 % in October/November. In November 1990 the lactating females ate 60 % more dry matter than non-lactating females.
Table 6.6 Annual foliage intake by female koalas in 1991 (areas under the curves in Figure 6.2).

<table>
<thead>
<tr>
<th></th>
<th>Intake (g DM)</th>
<th>Increase (%)* (g.kg(^{-0.67}).d(^{-1}))</th>
<th>Average</th>
<th>Peak lactation</th>
<th>Greatest</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL</td>
<td>70,675</td>
<td>21,304</td>
<td></td>
<td>20</td>
<td>27</td>
</tr>
<tr>
<td>L</td>
<td>84,935</td>
<td>25570</td>
<td></td>
<td>27</td>
<td>37</td>
</tr>
</tbody>
</table>

1 Increase over the non-lactating level.

6.3.5 Resting metabolic rate

No scaling relationship was statistically significant among the present data, untransformed or logarithmically transformed. However data from the largest sample, non-lactating, fed females, scaled with an exponent of 0.785.

\[
\text{RMR (kJ.d}^{-1}\) = 179.82 M(kg)^{0.785} \\
\text{r}^2 = 0.404 \text{ (P}>0.05, \text{n} = 6, \text{SE of coefficient} = 0.476).
\]

This at least suggests that Dawson and Hulbert's (1970) published exponent (0.737) may be appropriate for intraspecific scaling in koalas.

The RMR of fed, lactating, female koalas (244 ± 32 kJ.kg\(^{-0.737}\).d\(^{-1}\)) was greater than that of fed, non-lactating, female koalas (197 ± 30 kJ.kg\(^{-0.737}\).d\(^{-1}\)) (ANCOVA, mass as covariate, P=0.038), but there were no significant differences in fasted RMR's (P=0.916) (Table 6.7).

If non-lactating and lactating RMR's are pooled, an effect of specific dynamic action is apparent. Fed RMR's were higher than the fasted level (mean difference = 165 ± 155 kJ.d\(^{-1}\). Paired t-test, P=0.031). However, within both non-lactating and lactating groups, considered separately, there was no such effect (P=0.259, P=0.104 respectively). The difference between fed and fasted levels was greater in lactating females (mean difference = 237 ± 144 kJ.d\(^{-1}\)) than in non-lactating females (mean difference = 110 ± 158 kJ.d\(^{-1}\)).
<table>
<thead>
<tr>
<th>Status</th>
<th>Mass (kg)</th>
<th>O₂ consumption (ml.g⁻¹.h⁻¹)</th>
<th>RMR kJ.d⁻¹</th>
<th>RMR kJ.kg⁻⁰.⁷³.d⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL (fed)¹</td>
<td>5.82 ± 0.95</td>
<td>0.249B ± 0.039</td>
<td>721B ± 141</td>
<td>197 ± 30</td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L (fed)</td>
<td>6.20 ± 1.07</td>
<td>0.303A ± 0.037</td>
<td>943A ± 195</td>
<td>244 ± 32</td>
</tr>
<tr>
<td>(5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NL (fast)</td>
<td>5.10 ± 0.41</td>
<td>0.254 ± 0.026</td>
<td>615C ± 77</td>
<td>185 ± 19</td>
</tr>
<tr>
<td>(5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L (fast)</td>
<td>5.25 ± 0.76</td>
<td>0.250 ± 0.005</td>
<td>625C ± 104</td>
<td>184 ± 11</td>
</tr>
<tr>
<td>(3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Fed and Fast refer to the absorptive status of the koalas. Fasted koalas were fasted for 24 hours, whereas food was available to the fed koalas until the beginning of the measurement. L are lactating and NL are non-lactating females.

Within a column, means bearing different superscripts differ significantly (P< 0.05).

6.4 DISCUSSION

6.4.1 Equilibration of body water tracers

The equilibration time for use in the rest of the study was taken to be 120 minutes because the tracer concentration had reached a minimum in 75% of the koalas (6/8) at this time, as opposed to only 12.5% (1/8) at 60 minutes. However, the stability of the tracer concentration in the blood between 60 and 180 minutes indicates that precise timing of the equilibration period is unnecessary in koalas.

6.4.2 Body size and composition

Body water content of free-living koalas in this study calculated from dilution of $^3$H₂O, 68-77% was similar to reported levels in free-living koalas (Degabriele et al., 1978; Nagy and Martin, 1985). The water content calculated from the dilution of $^{18}$O was greater than that calculated from $^3$H dilution. This is unusual, as the body water space calculated from $^3$H dilution is usually about 3% higher than that from $^{18}$O (Nagy, 1980; Nagy and Costa, 1980; Schoeller et al., 1986). The difference in water space calculated for the two isotopes is usually assumed to indicate the presence of a pool of rapidly exchangeable hydrogen other than in body water (Nagy, 1980; Nagy and Costa, 1980). The reversal of the difference in koalas may indicate the absence of this hydrogen pool or the existence of a similar oxygen pool. Despite $^3$H₂O dilution overestimating body water, comparison between groups within this study should still be valid, as the same technique was used throughout.
Although there were no consistent differences in body mass or composition between lactating and non-lactating females, there were several instances in which the body composition differed. In August/September 1990 and May 1991 the lactating females had lower percentage body water, and in October/November and December 1991 higher percentage body water, than the non-lactating females. As the proportion of body mass which is water (percentage body water) is inversely proportional to the fat content (Searle, 1970; Green and Eberhard, 1983) this could be interpreted as storage of fat in lactating females during early to mid-lactation and the depletion of those fat stores late in lactation. However, there were no corresponding temporal changes in total body solids. This suggests that any storage of fat during early lactation by koalas is probably minimal. It is more likely that the differences in body water between lactating and non-lactating koalas reflect a greater mass of gut contents in lactating females late in lactation, as found in rats (Peters and Krynen, 1966) and bandicoots (Hulbert and Gordon, 1972).

The apparent lack of fat storage and utilization during lactation in the koala may be symptomatic of their generally lean body composition (Degabriele et al., 1978) and the fine energy balance imposed by the nutritional limitations of a diet of Eucalyptus foliage (Cork and Sanson, 1990), although Mitchell et al. (1988) note large subcutaneous fat deposits in three non-lactating koalas and, under maintenance conditions at least, koalas are well able to meet energy demands from their Eucalyptus diet (Cork et al., 1983). The energetic cost of vertical movement (Janzen, 1978) or strength of terminal branches (Grand, 1978) may preclude the extra weight of fat deposits. New growing foliage at the end of branches would be more difficult for a heavy-bodied arboreal folivore to access, although it seems unlikely that "fat" female koalas could not forage effectively when male koalas are normally much heavier (Lee and Martin, 1988) and are able to do so.

Among eutherians, maternal stores (Section 1.4.3) laid down during early gestation contribute considerably to meeting the demands of lactation in small rodents (Randolph et al., 1977; McLure, 1987; Weiner, 1987a), rabbits (Partridge et al., 1986), ungulates (Thomas, 1982; Tyler, 1987), bears (Ramsay and Dunbrack, 1986; Watts and Hansen, 1987) and pinnipeds (Fedak and Anderson, 1982; Bonner, 1984; Ortiz et al., 1984; Costa et al., 1986; Oftedal et al., 1987). Due to the high efficiency of utilization of stored energy, storage and later use of energy effectively spreads the demands of lactation out over time, thus minimizing the need for increases in food intake at times of peak demand (Pond, 1984; Partridge et al., 1986).
Some other marsupials store energy in the form of fat reserves during early lactation, and use these reserves during peak and late lactation. In the bandicoot, *Isoodon macrourus*, maternal body mass increases 10-20% during the first half of lactation, then decreases back to the mass at parturition during late lactation (Merchant, 1990), due to storage and use of fat reserves. Similar patterns of energy storage and use have been observed in *Trichosurus vulpecula* (Kennedy and Heinsohn, 1974; Bell, 1981), *Sarcophilus harrisii* (Nicol, 1978), *Dasyurus viverrinus* (Green and Eberhard, 1983) and *Petrogale inornata* (Kennedy and Heinsohn, 1974). Lactating common ringtail possums, *Pseudocheirus peregrinus*, undergo more pronounced fluctuations in condition than do koalas, as indicated by percentage body water and total body solids, or than male ringtail possums, with good condition in the early stages (phases 1 and 2) of lactation and poor condition in late lactation (phase 3) (Munks, 1990). However, Munks (1990) suggested that the magnitude of fat storage in *Pseudocheirus* was small, and that part of the increase in body solids during early lactation was due to an increase in mass of the mammary gland. Like koalas, tammar and Bennett's wallabies, and probably most macropodids, store and subsequently utilize little body fat, relying entirely on increases in intake to support the demands of lactation (Loudon, 1987a; Cork, 1991).

### 6.4.3 Field Metabolic Rate

**6.4.3.1 Potential errors with the use of isotopic tracers**

The accurate measurement of FMR and water flux is dependent on the validity of a number of assumptions about the behaviour of the tracers in vivo (Lifson and McLintock, 1966).

1. The body water volume remains constant during the measurement period.

This assumption was not tested in this study, rather the body water percentage was assumed to be constant for the calculation of body water at recapture. The equations used to calculate FMR and water flux allowed for small changes in body water volume, assuming that the change was linear over the measurement period (Nagy, 1983). Use of this equation results in small errors (< 5 %) in the calculated FMR (Nagy, 1980) and water flux (Nagy and Costa, 1980). Moreover, body mass changes over the measurement periods were small (< 1 %) and so it is unlikely that body water volume changes were a serious source of error in the calculation of FMR and water flux.
2. The flux rates of water and CO$_2$ are constant throughout the measurement period. Violation of this assumption can lead to large errors (14%) in calculated water fluxes (Nagy and Costa, 1980). However, this is an extreme case and more commonly the error is < 5% (Nagy and Costa, 1980). In the case of animals like the koala where water intake in food is frequent and gut-fill maintained at a nearly constant level, the error due to fluctuations in flux rate is probably low. If water efflux and influx vary in parallel then the error is zero and the calculated flux is the average of the flux rate (Lifson and McLintock, 1966). FMR calculations should not be affected by fluctuations as they will effect the $^{18}$O and $^3$H label approximately equally, leading to a constant difference (Nagy, 1980).

3. The tracers label only body water and CO$_2$.

$^3$H$_2$O can be rapidly incorporated in other compounds than water, leading to overestimation of body water and error in calculated fluxes (Lifson and McLintock, 1966; Holleman and Dieterich, 1975; Nagy and Costa, 1980; Nagy, 1980). Use of H$_2^{18}$O dilution to estimate body water removes much of this error (Nagy, 1980). In the present study, tritiated water space was used to estimate body water because body water estimates from $^3$H$_2$O were made in all the measurement periods for all the animals, whereas estimates using H$_2^{18}$O were made in only a subset of the measurements. However, this possible source of error in the calculated flux rates was unlikely to invalidate the major aim of the study; to compare flux rates in lactating and non-lactating female koalas.

4. Isotopes leave the body only in the form of water and CO$_2$.

Some tracer does leave the body in molecules other than H$_2$O and CO$_2$, but in low proportions leading to little error (< 1%, Lifson and McLintock, 1966; Nagy and Costa, 1980; Nagy, 1980). Tracers may have been incorporated into milk components and exported as such, but the quantity and therefore error associated is not known.

5. The specific activity, or concentration, of tracers leaving the body are equivalent to that in body water.

Because the isotopic tracers used in these measurements are heavier than $^1$H and $^{16}$O, they may be fractionated during metabolic processes. One potential source of this error is respiratory water loss. It is possible to correct for fractionation if the amount of loss through that avenue is known and the type of fractionation occurring is known. In practice this is not possible. The error in calculated water flux due to fractionation is about 4.7% in an animal whose entire water loss is by evaporation (Nagy and Costa, 1980). Nagy (1980) found that fractionation had no significant effect on FMR
measured with $^3$H$_2$H$^{18}$O, while Lifson et al. (1955) found approximately 10 % error in measurements done with $^2$H$_2$H$^{18}$O.

Haggarty et al. (1988) have proposed a variation of the doubly-labelled water method for measurement of FMR. This involves use of three labels (e.g.$^3$H$^2$H$^{18}$O) to measure the extent of and to correct for isotope fractionation. They calculate that the error in this method is around 0.3 %. Their method could not generally be used in this study as deuterium was already being used for the estimation of milk production.

This assumption is also violated when digesta retention is less than time for equilibration, such as in some frugivores, during diarrhoea, or in animals feeding young by regurgitation (Nagy and Costa, 1980), but digesta retention in koalas is long enough to avoid that source of error (Chapter 7).

6. Water and CO$_2$ do not enter the body through the skin or respiratory surfaces.

Violation of this assumption can occur under certain circumstances, such as in burrows where CO$_2$ levels and humidity can be high, and leads to large errors in the measured FMR and water flux (-96 to +81 % dependent on conditions, Nagy and Costa, 1980; Nagy, 1980). However, if the ambient air is low in or free of CO$_2$ and the ratio of $^3$H$_2$O in ambient air to that in the animal is similar to the ratio of ambient H$_2$H$^{18}$O to that in the animal, such as generally occurs above ground, then the error should be small (Lifson and McLintock, 1966). As koalas do not nest, errors due to cutaneous and respiratory water and CO$_2$ absorption are probably small.

Recycling of isotopes from mother to young could potentially cause large errors in calculated fluxes (Section 5.4.1; Baverstock and Green, 1975; Friedman and Bruno, 1976; Dove et al., 1989). However, errors due to isotope recycling in the koala are likely to be small as, when the young are in the pouch and recycling could occur, they are small compared with the mother (Section 5.4.1; Dove et al., 1989).

### 6.4.3.2 Validation of isotopic water tracer methods

CO$_2$ fluxes calculated by the doubly-labelled water method are usually within ± 8 % of the CO$_2$ production measured directly (Nagy, 1989). Validations of the use of $^3$H$_2$O have found it to be accurate to within 7 % (Nagy and Costa, 1980), with similar accuracy in the use of $^2$H$_2$O (8 %, Lifson and McLintock, 1966).

In the greater glider, *Petauroides volans*, the doubly-labelled water technique (with low levels of isotope enrichment as in this study) underestimated CO$_2$ production by an average of 8.3 % Nagy et al. (1990), and $^3$H$_2$O flux overestimated water intake by
an average of 8.5 % in the ringtail possum, *Pseudocheirus peregrinus*, (Munks, 1990). However, calculation of metabolic water production is difficult for folivores consuming *Eucalyptus* foliage (Section 6.2.1.5) and may have been overestimates, which would make the use of $^3$H$_2$O more accurate than 8.5 % (Munks, 1990).

6.4.3.3 Field Metabolic Rate

On the basis of the recent validation of the doubly-labelled water technique in greater gliders (Nagy *et al.*, 1990) it is likely that the field metabolic rates measured in this study are accurate to within approximately 8 % of the true FMR. Theoretically then, it is possible to detect differences of about 10 % between mean values.

Field metabolic rates measured in koalas in this study were similar to the previously reported value for female koalas (Nagy and Martin, 1985). The CO$_2$ production measured by Nagy and Martin (1985) for non-lactating females on the Victorian coast in August/September 1979, 0.503 ml.g$^{-1}$.h$^{-1}$, is slightly lower than that found at Nowendoc in August/September 1990, 0.570 ml.g$^{-1}$.h$^{-1}$. If the seasonal trend toward high energy use at Nowendoc is due to an increase in thermoregulatory requirements, it seems reasonable that this increase should be higher inland at an altitude of 900-1000 m. Nagy and Martin (1985) reported temperatures of 10-15°C, barely below the winter lower critical temperature in koalas of 10°C (Degabriele and Dawson, 1979), whereas during the measurement period at Nowendoc ambient temperatures ranged from -2 to 19°C (unpublished Forestry Commission data).

Using Degabriele and Dawson's (1979) whole body conductance value of 1.29 W.°C$^{-1}$.m$^{-2}$ and relationship between mass and surface area in koalas (Equation 6.5),

\[ SA(\text{cm}^2) = 9.9 \ M(\text{g})^{0.67} \quad \text{Equation 6.5} \]

it is possible to calculate that an average difference in ambient temperature of 6°C would result in heat loss equal to the the observed difference in mass-specific FMR between Nagy and Martin's (1985) and the August/September 1990 measurement of this study, well within the range of temperature differences observed.

Alternatively, if the intraspecific scaling exponent appropriate for koalas is 0.576, as used here, almost all (only 20 kJ.d-1 residual) of the mass-specific difference between FMR's in the two studies is due to change in mass-specific FMR associated with the difference in koala body mass between the two studies (6.03 vs 7.8 kg).
The predicted FMR for a 6 kg marsupial is 1771 kJ.d\(^{-1}\), or 631 kJ.kg\(^{-0.576}\).d\(^{-1}\) (Nagy, 1987), well within the range of FMR's measured in this study. However, the predicted FMR for an average eutherian of the same weight is 3950 kJ.d\(^{-1}\) (Nagy, 1987). The FMR of koalas ranges from 32 to 56 % of that of the same sized eutherian. Compared with average eutherians, koalas are conservative in energy use, but are similar to other marsupials. Thus, low FMR of koalas cannot be viewed as an adaptation to arboreal folivory, but be a preadaptation (Degabriele and Dawson, 1979; Cork and Sanson, 1990). Hume et al. (1984) proposed that energetic conservation in folivores is most likely to be manifested as a relatively low ratio of FMR to SMR. There is generally a trend to lower FMR:SMR ratios in the folivores (Table 6.8), but Cork and Sanson (1990) demonstrated that this ratio in koalas is no less than in similar sized terrestrial marsupials.

There was no significant increase in FMR in lactating females at peak lactation (August/September 1990). The small increase observed was only 6 % of the non-lactating FMR. This suggests that koalas may compensate for the energetic demands of lactation by reducing other components of their energy budget. This is discussed further in Section 6.4.6.

Surprisingly, there was an increase in maternal FMR early in lactation (January 1991, <100 days, see Chapter 5). Energy requirements for milk production were low at this stage (Figure 5.12), so the increase in FMR cannot have been directly due to the immediate demands of milk production. However, lactation results in changes in the size and activity of a number of body organs (Hanwell and Peaker, 1977; Vernon and Flint, 1984), so it is possible that the increased FMR of lactating koalas at this time reflects the synthesis of tissues necessary later in lactation, especially the digestive tract. Several studies of small herbivores have found increases in size and absorptive capacity of the gut in response to nutritional or cold stress (Moss, 1983; Gross et al., 1985; Green and Millar, 1987; Hammond and Wunder, 1991; Loeb et al., 1991; Toloza et al., 1991) or reproductive demands (Myrcha, 1964, 1965). Foley and Cork (1992) suggested that the plasticity in gut size and absorptive capacity observed in these studies may be important in allowing small herbivores to meet the demands of reproduction from a fibrous diet. As well as digestive tissue, it is also possible that liver tissue and enzyme systems may be developed and activated in preparation for increases in digestion and detoxification of allelochemicals associated with increases in food intake later in lactation, although there is no evidence in the literature that this would occur so long before the large increases in intake.
Trends among mammals in changes to FMR during lactation are not consistent (Table 6.8). However, there are few data available to date.

Kenagy et al. (1990) found no significant difference in FMR between non-lactating and lactating golden-mantled ground squirrels, and very little increase in FMR with increase in litter size from one to five. They concluded that increases in FMR related to lactation were small related to the energy contained in milk, and could be largely attributed to the heat increment of the increased food intake associated with milk production.

The greatest reported increases of FMR in lactating females over the non-lactating were in the small insectivores *Antechinus swainsonii* and *Eptesicus fuscus* (Table 6.8). However it is likely that in these cases the observed increase in FMR was not due primarily to the energetic demands of lactation but largely to an increase in cost of foraging, the costs of extra foraging flight in *Eptesicus* (Kurta et al., 1990) and costs associated with carrying the growing litter in *Antechinus*. Similarly, in the smaller *Antechinus stuartii* there is also an increase in FMR during lactation, but this falls sharply once the young are left in a nest (H. Crowley, pers. comm.), hence the low increase in FMR during late lactation (Table 6.8). Racey and Speakman (1987) found an increase of 30 to 40 % in the FMR of lactating bats, *Plecotus auritus*, over the non-lactating level. However, this did not reflect the full energetic requirements of lactation in these bats as they compensated for lactational energy expenditure by reducing their expenditure on thermoregulation, becoming heterothermic (Racey and Speakman, 1987). This makes them difficult to compare with homeothermic mammals. In *Plecotus*, as with *Antechinus*, the energy costs of increased foraging make up a large proportion of the increase in FMR (Racey and Speakman, 1987).

The costs of locomotion and foraging could also be expected to increase in koalas carrying young, especially late in lactation when the young can be 40 % of the maternal mass. That there was no observable increase strengthens the theory that koalas compensate for the energy demands of lactation (Section 6.4.6).
Table 6.8 Field Metabolic Rate and increases during lactation in mammals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mass (kg)</th>
<th>FMR* kJ.kg$^{-0.58}$.d$^{-1}$</th>
<th>Increase during lactation %</th>
<th>FMR:SMR</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phascolarctos cinereus</em></td>
<td>5.83-6.25</td>
<td>455-777</td>
<td>E +27</td>
<td>1.8-3.1B</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>7.8</td>
<td>611</td>
<td>E -13F</td>
<td>2.4B</td>
<td>2</td>
</tr>
<tr>
<td><em>Pseudocheirus peregrinus</em></td>
<td>0.953</td>
<td>644</td>
<td>L +19</td>
<td>2.1-2.9</td>
<td>3</td>
</tr>
<tr>
<td><em>Petauroides volans</em></td>
<td>0.934</td>
<td>512</td>
<td></td>
<td>2.7</td>
<td>4</td>
</tr>
<tr>
<td><em>Gymnobelideus leadbeateri</em></td>
<td>0.129</td>
<td>735</td>
<td>E 0</td>
<td>5.8</td>
<td>5</td>
</tr>
<tr>
<td><em>Petaurus breviceps</em></td>
<td>0.112</td>
<td>540</td>
<td>E 0C</td>
<td>3.8</td>
<td>6</td>
</tr>
<tr>
<td><em>Antechinus swainsonii</em></td>
<td>0.054</td>
<td>675</td>
<td>L +72</td>
<td>4.5E</td>
<td>8</td>
</tr>
<tr>
<td><em>Antechinus stuartii</em></td>
<td>0.028</td>
<td>726</td>
<td>L + 11</td>
<td>1.7</td>
<td>9</td>
</tr>
<tr>
<td><em>Bradypus variegatus</em></td>
<td>4.22</td>
<td>214</td>
<td>+33C</td>
<td>2.0</td>
<td>10</td>
</tr>
<tr>
<td><em>Alouatta palliata</em></td>
<td>6.03</td>
<td>878</td>
<td>-30D</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Spermophilus saturatus</em></td>
<td>0.232</td>
<td>666</td>
<td>+17F</td>
<td>2.6</td>
<td>11,12</td>
</tr>
<tr>
<td><em>Eptesicus fuscus</em></td>
<td>0.017</td>
<td>698</td>
<td>+ 88</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td><em>Myotis lucifugus</em></td>
<td>0.008</td>
<td>408</td>
<td>0</td>
<td></td>
<td>14</td>
</tr>
</tbody>
</table>

*Values are for non-lactating females
E early lactation
L late lactation
A During peak lactation
B Calculated using the RMR measured in non-lactating females in this study.
C Based on comparison with one non-lactating female only.
D Based on comparison with one lactating female only.
E Value for *Antechinus stuartii* from Nagy et al. (1978)
F Not statistically significant.
N.B. The FMR of the eutherians in Table 6.8 have been scaled to Mass$^{0.576}$ in order to compare them with marsupials, despite this being an inappropriate scaling factor for eutherians (Nagy, 1987).

1 This study.
2 Nagy and Martin (1985)
3 Munks (1990)
4 Foley *et al.* (1990)
5 Smith *et al.* (1982)
6 Nagy and Suckling (1985)
7 Lee and Nagy (unpublished manuscript) cited in Lee and Cockburn (1985)
8 Green *et al.* (1991a)
9 Nagy and Montgomery (1980)
10 Nagy and Milton (1979a)
11 Kenagy *et al.* (1990)
12 Kenagy (1987)
13 Kurta *et al.* (1990)
14 Kurta *et al.* (1989)
6.4.4 Water and Food Intake

The range of water intake in the present study (37-60 g.kg\(^{-1}\).d\(^{-1}\)) encompasses the water intake measured by Nagy and Martin (1985) of 44 g.kg\(^{-1}\).d\(^{-1}\) in free-living koalas and by Eberhard (1972) in captive koalas. However, Degabriele et al. (1978) report water intakes two to three times those in the present study, in similar sized free-living koalas. There is no apparent reason for the large difference between Degabriele et al.'s (1978) values and those from this study, unless their study animals drank free water.

Intake of dry matter from foliage by non-lactating female koalas in Victoria (56 g.kg\(^{-0.67}\).d\(^{-1}\), Nagy and Martin, 1985) was within the range of intake by non-lactating females in this study (39-66 g.kg\(^{-0.67}\).d\(^{-1}\)).

Apart from the possible errors associated with the use of \(^3\)H\(_2\)O as a body water tracer, the measurements of food intake in this study are open to errors associated with some of the assumptions made in the calculations. First, I have assumed that no free water is consumed. This is probably sound in most cases, as koalas are able to maintain water balance without taking in free water (Degabriele et al., 1978), and there are rarely pools of water available in the trees. However, during rain, and heavy frost and dew, water coating the leaves is available to koalas. Munks (1990) found from her calculation of food and water intake in ringtail possums, that lactating females took in a greater proportion of their water as free water, presumably dew, than non-lactating females. To an extent her result is an artifact of her method of calculation. She assumed that food energy intake was equal to energy expenditure as measured by FMR. The energy exported in milk by lactating females does not contribute to CO\(_2\) production and thus to FMR. Consequently she underestimated food energy intake, and hence the preformed water intake in food.

There were few days of rain or frost during the measurement periods (unpublished observations; unpublished Forestry Commission records). There were only four days of light rain over the five periods in which food intake differed between lactating and non-lactating koalas. The period August to November 1991 was particularly dry, with half the precipitation of the same period in 1990. I have never observed koalas licking water from the surface of leaves and conclude that free water intake in this form is low, and unlikely to contribute to errors in the comparison of lactating and non-lactating koalas.

Another possible source of error in my food intake calculations is the sampling and determination of the foliage water content. The samples were taken from the canopy...
of trees in which the koalas were found at capture. Where new leaves (tips) and mature leaves were found together in sufficient quantities the water content of each was determined separately. However, for the purposes of calculation I assumed that the water content of foliage consumed was the average of tip and mature leaves. If koalas consumed large quantities of foliage with different water content, either from other trees or by selecting for a specific age class of leaves, the food intake calculated may be wrong. Any error from this source is most likely to be significant when new leaves are abundant. There was little new foliage available to the koalas from May to November 1991, with the flush of leaf growth beginning after rains in December (Figure 8.1). Tips were available throughout 1990 (Figure 8.1). Because of the limited availability of tips during the periods in 1991 (August/September to November) in which food intake was greater in lactaters than non-lactaters, it is unlikely that a differential selectivity for tips between the groups caused large errors in calculated food intakes. However, in November 1990 and December 1991, when tips were abundant (Figure 8.1), the food intake results may possibly have been biased by differential selectivity for tips between the two groups of females, although there was no evidence for differences in diet selectivity between lactaters and non-lactaters (Chapter 8).

It is likely that there were some errors in the assumptions involved in calculating food intake, as the energy balance (Table 6.5) was occasionally negative, even though the koalas were not losing body mass or solids. This was most evident in May 1991, when it rained lightly during most of the measurement period, and may have been caused by sampling of wet leaves and therefore overestimation of their water content and consequently underestimation of food intake. However, the data are likely to be adequate for the purposes of comparison within this study.

Lactating female koalas had greater food and water intake in late lactation than non-lactating females (Figure 6.2). At peak lactation in 1991 the differences in food and water intake were 27 and 20 % respectively, and in 1990 they were 27 and 17 %, although not statistically significant because of the greater variability and smaller sample size than in 1991. The greatest difference in food and water intake between lactating and non-lactating koalas occurred in November 1990.

The range of increases in food (27 %) and water (17-20 %) intake at peak lactation were low compared to the range of increases measured in other free-living mammals (Table 6.9). Lactating female koalas consumed 20 % more foliage dry matter than non-lactating females annually, similar to the 24 % annual increase in consumption attributable to the demands of reproduction in golden-mantled ground squirrels,
Spermophilus saturatus (Kenagy et al., 1989a). However, unlike the koala, in the ground squirrel the extra consumption and reproduction are constrained to the 4-5 month period of activity (Kenagy et al., 1990), and at the peak of lactational energy demand is probably closer to a 90-100% increase. Increases in food intake at peak lactation in koalas were only a third of those in tammar and Bennett's wallabies, although those wallabies were captive with abundant food (Loudon, 1987a; Cork, 1991).

Table 6.9 Increases in the intake of food and water by free-living lactating mammals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Lactational Stage</th>
<th>Water (%)</th>
<th>Food (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phascolarctos cinereus</td>
<td>L</td>
<td>+17-20</td>
<td>+27</td>
<td>1</td>
</tr>
<tr>
<td>Pseudocheirus peregrinus</td>
<td>E</td>
<td>+3</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Trichosurus vulpecula</td>
<td>L</td>
<td>+12</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Gymnobelideus leadbeateri</td>
<td>E</td>
<td>-13</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Petaurus breviceps</td>
<td>E</td>
<td>+54</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Petrogale inornata</td>
<td></td>
<td>+20</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Isoodon macrourus</td>
<td>E</td>
<td>+10-40</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Antechinus swainsoni</td>
<td>L</td>
<td>+93</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Antechinus stuartii</td>
<td>L</td>
<td>+74</td>
<td>+74</td>
<td>8</td>
</tr>
<tr>
<td>Dasyurus viverrinus</td>
<td>E</td>
<td>0</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>L</td>
<td>+59</td>
<td>+60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bradypus variegatus</td>
<td></td>
<td>+35</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Spermophilus saturatus</td>
<td>L</td>
<td>+21</td>
<td>+90</td>
<td>11</td>
</tr>
<tr>
<td>Marmota flaviventris</td>
<td></td>
<td>+81</td>
<td>+92</td>
<td>12</td>
</tr>
<tr>
<td>Eptesicus fuscus</td>
<td>L</td>
<td>+143</td>
<td>+115</td>
<td>13</td>
</tr>
<tr>
<td>Myotis lucifugus</td>
<td>L</td>
<td></td>
<td>+78</td>
<td>14</td>
</tr>
</tbody>
</table>

E denotes early lactation; phases 2a and 2b (Tyndale-Biscoe and Janssens, 1988)
L denotes late lactation, including peak; phase 3 (Tyndale-Biscoe and Janssens, 1988)

1 This study.
2 Munks (1990)
3 Kennedy and Heinsohn (1974)
4 Smith et al. (1982)
5 Nagy and Suckling (1985)
6 Hulbert and Gordon (1972)
8 Green et al. (1991a)
9 Green and Eberhard (1983)
10 Nagy and Montgomery (1980)
11 Kenagy et al. (1990)
12 Melcher et al. (1989)
13 Kurta et al. (1990)
14 Kurta et al. (1989)

6.4.5 Resting Metabolic Rate

The fasted-resting metabolic rate of non-lactating koalas in the present study was 20 % greater than the previously measured basal metabolic rate (BMR) for koalas (Degabriele and Dawson, 1979), and 90 % of the expected BMR for marsupials
The fed-resting metabolic rate of lactating females was 20% higher than the expected BMR for marsupials. Degabriele and Dawson (1979) made most of their measurements in summer, while those in this study were made in early spring. The difference in metabolic rate between this study and Degabriele and Dawson (1979) may be partly due to seasonal differences. Additionally, Degabriele and Dawson (1979) did not indicate the time for which koalas were fasted, so differences may be due to methodological differences.

The RMR of lactating koalas was greater than that of non-lactating female koalas. The difference in RMR (47 kJ.kg\(^{-0.737}\).d\(^{-1}\), or 176 kJ.d\(^{-1}\) for a 6 kg female) was probably due to the extra requirements of milk production and digestion associated with lactation.

Increases in RMR during reproduction have been recorded in other mammals. RMR increased during lactation in several small marsupials and eutherians (Fleming et al., 1981; Thompson and Nicoll, 1986; Nicoll and Thompson, 1987). RMR increased in species with low BMR but RMR increased much less, or not at all, in species with BMR close to Kleiber's prediction (Kleiber, 1975; Nicoll and Thompson, 1987). Nicoll and Thompson (1987) interpreted this pattern to mean that the BMR predicted by Kleiber's relationship (Kleiber, 1975) is close to an optimal level for mammalian reproduction. Presumably the increases in RMR as lactation progressed were due to the increasing energetic costs of milk production and SDA. It is not clear from their data whether the increases in RMR they observed were sufficient to account for the energy required to produce milk and digest food, so it is hard to know if compensation occurs in Monodelphis. However, in species that show no increase in RMR during lactation, compensation for the energy requirements of reproduction by reduction of other components of the energy budget must occur.

**6.4.6 Energy budget of a 6 kg koala at peak lactation: Is there energetic compensation?**

If there was no compensation for the energy requirements of lactation, milk production was 65% energy efficient (ARC, 1980), the heat increment of digestion (SDA) was 15% (Kleiber, 1975) and all energetic requirements were met from current nutrition, then the FMR and RMR of a 6 kg lactating koala at peak lactation in 1990 should have been 420 kJ.d\(^{-1}\) greater than that of a 6 kg non-lactating koala (Table 6.10). However, FMR was not increased, and RMR increased by only 42% of that expected. If variances remained as measured, an increase of 420 kJ.d\(^{-1}\) would have been statistically significant (P=0.005). Therefore, there was compensation for reproductive energy expenditure by a reduction in other components of the energy
budget, partly in RMR and partly in the non-RMR portion of FMR, activity and thermoregulation. Alternatively, if efficiency of production of milk was higher than 65 %, compensation may not have been necessary.

Food intake of lactaters was 15 g.kg-0.67.d-1 (50 g.d-1 for a 6 kg female) greater than that of non-lactaters, yielding 495 kJ.d-1 extra metabolizable energy, about equal to the export of energy in milk. Thus, the energy exported in milk was supplied by increased food intake, while the energy required to synthesize that milk and process the extra food intake was compensated for by a reduction in some other component of their energy budget.

Table 6.10 Energy budget of a 6 kg koala at peak lactation in 1990, assuming no compensation for the energy requirements of reproduction1.

<table>
<thead>
<tr>
<th>Metabolizable energy (kJ.d-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMRNL</td>
</tr>
<tr>
<td>Milk production</td>
</tr>
<tr>
<td>Total requirements</td>
</tr>
<tr>
<td>for milk production</td>
</tr>
<tr>
<td>Milk synthesis</td>
</tr>
<tr>
<td>Extra food</td>
</tr>
<tr>
<td>SDA</td>
</tr>
<tr>
<td>SDA + Milk synthesis</td>
</tr>
<tr>
<td>Expected FMRL</td>
</tr>
<tr>
<td>Observed FMRL</td>
</tr>
<tr>
<td>Compensation</td>
</tr>
</tbody>
</table>

1 Assumptions: a) Milk production is 65 % efficient (ARC, 1980). b) SDA is 15 % of food metabolizable energy (Kleiber, 1975). c) All energy requirements are met by food energy intake.

Compensation for reproductive expenditure in koalas may be partly achieved by a reduction in some component of maintenance, as in humans and laboratory rats (Westerterp, 1977; Prentice and Whitehead, 1987), such as reduction in adipose tissue metabolism (Vernon and Flint, 1984; Roberts and Coward, 1984; Chan and Swaminathan, 1990), and/or thermoregulation (Westerterp, 1977; Racey and Speakman, 1987). As FMR of koalas at Nowendoc increased so markedly from summer to winter, and winter temperatures are well below their thermoneutral zone (Table 3.1), thermoregulatory expenditure probably accounted for a large proportion of the FMR at peak lactation. Reduction in thermoregulatory expenditure could be achieved in three ways. First, the conductivity of koala fur changes seasonally
(Degabriele and Dawson, 1979). If lactating koalas lowered their conductivity further than non-lactating koalas, their thermoregulatory requirements would be decreased. Second, reduction in thermoregulatory demands can be achieved by relaxing homeothermy. Reproducing female bats reduce their expenditure on thermoregulation (Racey and Speakman, 1987), by entering torpor during gestation and lactation. Their propensity to enter torpor is related to the food availability on the previous night (J. Speakman, pers. comm.), and results in energy requirements of only 80% of those predicted for normothermic bats during lactation. There is no evidence in the literature that koalas relax homeothermy (Degabriele and Dawson, 1979; Nagy and Martin, 1985), although they may often be observed basking. Third, a female koala carrying a furred juvenile at peak lactation effectively reduces the surface area over which she loses heat by huddling. Huddling would benefit both mother and young. For a 6 kg female with a 1 kg juvenile, huddling could reduce their combined exposed surface area by 15%, but that of the young alone by 32% and that of the mother by 10% (if we assume that that the surface area of the huddling pair is equal to that of a koala equal in mass to the sum of their masses, using Equation 6.5). Conductance of koalas is 1.29 W.°C⁻¹.m⁻², so a 10% reduction in surface area of the mother would lead to a 3.7kJ.d⁻¹.°C⁻¹ reduction in thermoregulatory expenditure, or about 108 kJ.d⁻¹ if the ambient temperature was 5°C (Table 3.1).

Compensation in maternal expenditure can also be achieved by a reduction in activity (Slonaker, 1925; Wang, 1925; Randolph et al., 1977), although activity in koalas is normally low, so that savings by reduction of activity could account for only a small part of the observed compensation (Chapter 9).

In summary, koalas increase their food intake by about 30% at peak lactation to meet the energy demands of reproduction. They don't use maternal stores to spread the load of lactation further, but they do compensate for the energy requirements of reproduction by reducing some other component of their energy budget, possibly thermoregulatory requirements. However, it is likely that the sources of compensation are multiple and individually small in magnitude. The following chapters investigate some of the possible consequences of increased intake and energy compensation. Increases in food intake can reduce time of retention of digesta in the gut and increase faecal nutrient losses; Chapter 7 investigates digesta retention during reproduction in female koalas while Chapter 8 considers diet composition. Reduction in activity compensates for reproductive expenditure in some mammals; Chapter 9 considers home ranges of koalas during reproduction.
CHAPTER SEVEN

PASSAGE OF DIGESTA THROUGH THE GUT OF FREE-LIVING KOALAS

7.1 INTRODUCTION


Cork and Warner (1983) reported mean retention times (MRT's) of 213 hours for a fluid digesta marker, and 100 hours for a particle marker in captive koalas. This indicates selective retention of the fluid marker in the gut. Two other marsupial arboreal folivores also selectively retain fluid digesta markers (Chilcott and Hume, 1985; Foley and Hume, 1987a). Cork and Warner (1983) proposed that the mechanism of selective retention in these marsupials is probably similar to that in the rabbit; i.e. by anti-peristaltic movements of the proximal colon, net secretion of water from blood into the proximal colon, and net absorption of water from the caecum (Bjornhag, 1972; Pickard and Stevens, 1972). Rubsamen et al. (1983) demonstrated water influx into the proximal colon of the greater glider, which is consistent with the proposed mechanism. Cork and Warner (1983) suggested that selective retention of fluid and fine particles (including bacteria), and more rapid excretion of large particles, allow koalas to minimize the gut-filling effect of their foliage diet while also minimizing faecal nitrogen losses associated with rapid throughput (Sperber, 1968).

In most herbivores, increases in intake are associated with decreases in MRT (Blaxter et al., 1956; Grovum and Hecker, 1973; Grovum and Williams, 1973a; Warner, 1981) and/or increases in gut capacity (Foley and Cork, 1992). Decreasing the MRT of digesta also decreases the digestibility of the diet in sheep and cattle (Blaxter et al., 1956; Grovum and Williams, 1973a), presumably by decreasing the time for bacterial fermentation. Some large hindgut fermenters reduce MRT and sacrifice digestibility, to maintain their intake of readily digestible components (Janis, 1976; Dierenfield et al., 1982; Duncan et al., 1990; Ilius and Gordon, 1992). Some small herbivores respond to increases in intake by increasing their gut capacity and absorptive ability.
Lactating koalas increased their foliage intake to meet most of the demands of reproduction (Chapter 6). Lactating females took in 23% more foliage dry matter than non-lactating females in the August/September 1991 measurement period, when MRT was measured. The increase in intake would be expected to lead to a decrease in MRT and/or an increase in gut capacity. Decreasing the MRT may reduce digestibility of the diet, hence increasing the foliage intake necessary to meet reproductive requirements.

The digestive response of lactating koalas to increased feed intake during reproduction is an important component of their reproductive strategy. This chapter investigates the MRT of digesta in koalas near the time of their peak reproductive requirements.

The rate of development of adult gut function in dependent juveniles is likely to control their ability to ingest foliage, thus placing a constraint on the lower limit of maternal energy transfer to the young during weaning. This chapter also investigates MRT during development of juvenile koalas.

### 7.2 MATERIALS AND METHODS

#### 7.2.1 Times of measurements

MRT's were measured concurrently with FMR and water intake during the August/September and October/November measurement periods in 1991 and January 1992. Digesta passage rates were compared in five lactating and four non-lactating females in August/September 1991, close to the peak of lactation (Chapter 6). Digesta passage rates were measured in juvenile koalas just after pouch exit at about 9 months (n=3, August/September 1991), during weaning at about 11 months (n=6, October/November 1991), after final weaning at 14 months (n=5, January 1992) and at 21 months (n=6, August/September 1991). The measurements of dependent and newly weaned juveniles were made on the same juveniles sequentially, but the 20-month old juveniles were the young from the previous season, measured immediately before the males dispersed.

#### 7.2.2 Preparation of markers

Commercially prepared cobalt-EDTA (LiCoEDTA) was used as the fluid marker and a chromium-cell wall mordant as the particle marker (Uden et al., 1980).
The particles were prepared by grinding chopped oaten hay through a 1000 μm screen. They were then wet sieved as described by Barboza (1989) and the size fraction 600-1180 μm collected and oven dried (50°C). The mordant was then prepared as described by Uden *et al.* (1980).

### 7.2.3 Dosage and collection of faecal samples

The prepared markers were mixed together (2 parts Cr-CWC and 1 part Co-EDTA, by weight) and individual doses of the marker mixture prepared (0.12 g.kg⁻¹ of the body mass at the previous capture). The marker doses were loaded into plastic 5 ml syringes with the distal end of the barrel cut off, labelled, and sealed with Parafilm until use.

Measurements of MRT were made concurrently with those of water intake and energy use; thus the capture and handling of the koalas was as described in Chapters 3 and 6. The digesta marker dose was given orally after the water and/or oxygen tracers had been administered and while the koalas were still sedated. The Parafilm was removed from the syringe and the contents moistened with water. The open end of the syringe was placed in the koala's mouth through the diastema and moved backward over the molars, then the digesta markers placed onto the back of the tongue by forcing them out with the plunger of the syringe. The syringe was withdrawn and the koala's mouth held closed until the markers were swallowed.

In general, 80-100% of the marker dose was administered to sedated koalas in this way. However, there were often losses of marker onto the fur and into the holding bag, and so the precise dose could not be determined.

After the isotope equilibration time (for the water intake or FMR measurements), the koala was released into the tree from which it had been captured. When it had climbed up to a settled position, usually in the canopy, a 6 m x 4 m x0.5 mm thick plastic sheet was spread on the ground underneath the koala and secured against wind with rocks or fallen branches. The plastic sheets were checked every six hours for the first four days, every eight hours for the next two days and at least every 12 hours thereafter until collection ceased at about 10 days. All the faeces on them were collected into plastic bags and stored frozen until analysis.

If the koala had moved during the collection interval it was radio-located and, after the faeces had been collected, the plastic sheet was moved to the new location. Because the koalas moved both within and between trees, it was not possible to collect the total faecal production of each animal. On several occasions it rained...
during collection periods and faeces found in pools of water were discarded as the fluid marker could potentially be leached out.

7.2.4 Analytical methods

Faeces were thawed and oven dried in their plastic bags at 50°C over a period of two weeks. The faecal pellets were broken up and mixed using a mortar and pestle, which was wiped clean between samples, then returned to the oven. Samples from the one animal were ground sequentially, to minimize problems with contamination from one sample to the next.

The faeces were taken from the oven and 1.5 g weighed into a conical flask, and 10 ml of nitric acid (HNO₃:H₂O, 1:1) added and mixed. The flask was placed on a warm hotplate in a fumehood, covered with a watch glass and refluxed without boiling for 15 min. Concentrated nitric acid (5 ml) was added and the slurry refluxed for 30 min. This step was repeated before displacing the watch glass slightly and allowing the solution to evaporate, without vigorous boiling, to about 5 ml. The solution was then cooled in air and distilled water (2 ml) and 30% hydrogen peroxide (3 ml) added, the watch glass replaced, and the flask warmed on the hot plate. After effervescence had subsided, 1 ml aliquots of H₂O₂ were added, leaving time for effervescence to subside between each, to a total of 10 ml H₂O₂. The solutions were then evaporated down to 5 ml as before, cooled, then washed into a volumetric flask (100 ml) and diluted to 100 ml. An aliquot (30 ml) of the resulting digestate was then filtered (Whatman 41 filter paper) into a plastic scintillation vial and stored at 5°C until analysis.

The acid/peroxide digested faecal solutions were analysed for content of Co and Cr using flame atomic absorption spectroscopy (AAS) or inductively coupled plasma optical emmission (ICPOES) spectroscopy by David Hill and Maree Emmett at the ANSTO Environmental Chemistry Laboratory, Lucas Heights NSW. These techniques detected Co and Cr at levels as low as 1-2 µg/g dry faeces.

7.2.5 Calculations

The natural logarithm of the faecal marker concentration (µg/g) was plotted against the midpoint of the time interval, after the marker dose, in which the faeces were collected. Data points with low concentration of cobalt due to rain during the collection were discarded, as were all points below background levels (2 µg/g).

After a lapse of time following dosing (i.e. transit time), marker concentrations increased rapidly to a peak, and then fell linearly. An exponential decay curve of the
form \( y = A e^{-kt} \) (Brandt and Thacker, 1958) was fitted to the curves after the peak by least squares linear regression of \( \ln(\text{concentration}) \) vs time. The turnover time of the major gut mixing compartment was calculated as \( 1/k \) (Grovum and Williams, 1973b).

In a number of cases the Cr curves showed a second linear component. These were determined by regression as a separate component (Brandt and Thacker, 1958; Foley and Hume, 1987a). However this component was always late (at least 100 hours after dosing), at Cr concentrations that were only 1-2% of peak concentrations and close to background, so it probably represented only a minor part of the marker dose (Foley and Hume, 1987a).

The transit time (time from dose to the first appearance of marker) was determined only from measurements where the first appearance of markers could be determined within a ten hour interval. Due to the difficulty of accurately estimating transit time in each animal, this measure of passage was not used for comparison between groups. Instead the slope of the regression (k) and its reciprocal \( 1/k \), the turnover time of the major compartment (Grovum and Williams, 1973b), were used. Turnover time estimated in this way closely approximates overall mean retention time of digesta in koalas because transit time is very short compared with retention in the hindgut (Cork and Warner, 1983).

The pool size of the major gut compartment available to the markers was estimated using the measured retention times for that compartment \( 1/k \) h and the estimated food intake (Chapter 6).

\[
\text{MRT} = \frac{\text{pool size}}{\text{intake}} \quad \text{Equation 7.1}
\]

Assuming that all the koalas masticated their food to the same extent, the fraction of intake corresponding to the fluid (Co) and 600-1180 \( \mu \)m particle (Cr) fractions can be termed \( X \) and \( Y \) respectively. Pool size\( _F \) and pool size\( _P \) denote the pool sizes of fluid and particle markers respectively and \( k_{Co} \) and \( k_{Cr} \) are the fractional turnover of Co and Cr markers respectively. Then,

\[
\frac{1}{k_{Co}} = \frac{\text{pool size}_F}{X \times \text{intake}} \quad \text{Equation 7.2}
\]

and

\[
\frac{1}{k_{Cr}} = \frac{\text{pool size}_P}{Y \times \text{intake}} \quad \text{Equation 7.3}
\]
Rearranging,

\[
\frac{1}{k_{Co}} \cdot \text{intake} = \frac{\text{pool size}_F}{X}
\]

and

\[
\frac{1}{k_{Cr}} \cdot \text{intake} = \frac{\text{pool size}_P}{Y}
\]

The proportions (X and Y) of the intake corresponding to the fluid and particle marker behaviour are unknown. However, if they are assumed to be constant between animals, the product of total intake and 1/k (Equations 7.4 and 7.5) can be used as indices of the pool sizes of the digesta markers in the major mixing compartment.

Estimates of overall average mean retention time for the digesta markers, for comparison with other studies, were made using the averages of \(k_{Co}\) and \(k_{Cr}\) for all measurements except the 9-month-old young, according to the method of Sakaguchi et al. (1987).

\[
\text{MRT} = \text{TT} + \frac{1}{k}
\]

where \(\text{TT}\) was calculated as the average transit time for all animals where transit time was known to within 10 h. Statistical comparisons were made using paired and unpaired versions of Student's t-test (Wilkinson, 1989).

7.3 RESULTS

7.3.1 Form of the faecal marker concentration curve

Faecal concentrations of the markers rose rapidly to a peak within 40 h of the dose, and then declined exponentially (e.g. Figure 7.1). The slope (\(k_{Cr}\)) of the linear decline in ln(Cr) was greater (P<0.001) than that of ln(Co), indicating that the fluid marker (Co) was retained longer than the particle marker (Cr) (Mean difference= 88 h, P<0.001). Separation of fluid and particle markers was evident from the earliest ages measured (at 9 months, P=0.007).

In some cases, as discussed above, a second linear component could be fitted to the decline in concentration of the particle marker (Cr). The average slope for this component was similar to that of the fluid marker (Co) (Mean difference=0, DF=14, P=0.953). However, because of the low concentrations of the Cr marker involved
(1-2 % of their peak, and close to background), this component was probably insignificant and has been ignored.

Figure 7.1 Faecal excretion of digesta markers in a koala (K68), showing the regression lines fitted to the terminal linear portion of the curves.

Co \[ y = 4.6 - 0.016 \times \] \[ r^2 = 0.99 \]

Cr \[ y = 6.7 - 0.055 \times \] \[ r^2 = 0.98 \]

7.3.2 Passage of markers in adult females

There were no statistically significant differences in the passage of digesta markers in the lactating and non-lactating adult females studied, even though foliage intake was 22 % greater in the lactating females (Chapter 6). Nevertheless, there was a trend \((P=0.055)\) toward a greater \(k\), and hence faster passage (ie. lower \(1/k\)) of the particle marker in the lactating than the non-lactating females (Table 7.1). One of the latter group (K14) was lactating in the early part of the season, but lost her young less than a month before the measurement of digesta passage. She may therefore not be representative of the non-lactating group. If she is removed from the analysis, the passage of the particle marker in lactaters is significantly faster than in non-lactaters \((P=0.042)\).
The difference in passage of fluid and particle markers within each koala (ie. $k_{Cr}-k_{Co}$) tended to be greater in lactaters ($0.051 \pm 0.016 \text{ h}^{-1}$) than non-lactaters ($0.028 \pm 0.014 \text{ h}^{-1}$), approaching significance ($P=0.056$) when K14 was excluded as being non-representative of the non-lactating group. These comparisons need to be repeated with greater sample sizes.

Table 7.1 Passage of digesta markers in adult female koalas.

<table>
<thead>
<tr>
<th>Koala</th>
<th>Status</th>
<th>Mass (kg)</th>
<th>$k_{Co}$ (h$^{-1}$)</th>
<th>$k_{Cr}$ (h$^{-1}$)</th>
<th>$1/k_{Co}$ (h)</th>
<th>$1/k_{Cr}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>L</td>
<td>5.82</td>
<td>0.0068</td>
<td>0.0838</td>
<td>148</td>
<td>12</td>
</tr>
<tr>
<td>27</td>
<td></td>
<td>6.80</td>
<td>0.0124</td>
<td>0.0590</td>
<td>81</td>
<td>17</td>
</tr>
<tr>
<td>34</td>
<td></td>
<td>5.88</td>
<td>0.0150</td>
<td>0.0465</td>
<td>67</td>
<td>21</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td>6.40</td>
<td>0.0104</td>
<td>0.0637</td>
<td>96</td>
<td>16</td>
</tr>
<tr>
<td>65</td>
<td></td>
<td>5.63</td>
<td>0.0086</td>
<td>0.0635</td>
<td>116</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>NL</td>
<td>5.95</td>
<td>0.0128</td>
<td>0.0437</td>
<td>78</td>
<td>23</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>6.03</td>
<td>0.0152</td>
<td>0.0531</td>
<td>66</td>
<td>19</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>5.60</td>
<td>0.0095</td>
<td>0.0446</td>
<td>106</td>
<td>22</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>5.53</td>
<td>0.0081</td>
<td>0.0162</td>
<td>124</td>
<td>62</td>
</tr>
<tr>
<td>Mean</td>
<td>L</td>
<td>6.10</td>
<td>0.0106</td>
<td>0.0621</td>
<td>102</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>(SD)</td>
<td>(0.48)</td>
<td>(0.0032)</td>
<td>(0.014)</td>
<td>(32)</td>
<td>(3)</td>
</tr>
<tr>
<td></td>
<td>NL</td>
<td>5.77</td>
<td>0.0114</td>
<td>0.0394</td>
<td>93</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>(SD)</td>
<td>(0.25)</td>
<td>(0.0032)</td>
<td>(0.016)</td>
<td>(26)</td>
<td>(20)</td>
</tr>
<tr>
<td>P*</td>
<td></td>
<td>0.26</td>
<td>0.735</td>
<td>0.055</td>
<td>0.691</td>
<td>0.241</td>
</tr>
<tr>
<td>Mean</td>
<td>All</td>
<td>5.96</td>
<td>0.0110</td>
<td>0.0520</td>
<td>98</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>(SD)</td>
<td>(0.41)</td>
<td>(0.003)</td>
<td>(0.018)</td>
<td>(28)</td>
<td>(15)</td>
</tr>
</tbody>
</table>

L = lactating
NL = non-lactating
* Probability of the difference between means in the column being due to chance alone, as measured by Student's t-test.

7.3.3 Capacity of the major gut compartment in adult female koalas

Although particle marker pool sizes did not differ between lactating and non-lactating females (Table 7.2), the fluid marker pool was 40% greater in lactaters than non-lactaters ($P=0.063$, $P=0.025$ if K14 is excluded). This difference may have been partly due to the slightly greater body mass of the lactating than the non-lactating koalas in this sample; the difference in fluid marker pool size per kilogram of body mass between the groups only approached significance ($P=0.052$).
Table 7.2 Digesta pool sizes in adult female koalas (means, SD in brackets).

<table>
<thead>
<tr>
<th>Pool size/Proportion of intake</th>
<th>Fluid (g)</th>
<th>Fluid (g.kg⁻¹)</th>
<th>Particle (g)</th>
<th>Particle (g.kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating</td>
<td>1117</td>
<td>185</td>
<td>199</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>(261)</td>
<td>(50)</td>
<td>(59)</td>
<td>(11)</td>
</tr>
<tr>
<td>Non-lactating</td>
<td>800</td>
<td>139</td>
<td>300</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>(163)</td>
<td>(34)</td>
<td>(148)</td>
<td>(28)</td>
</tr>
<tr>
<td>P*</td>
<td>0.063</td>
<td>0.154</td>
<td>0.271</td>
<td>0.253</td>
</tr>
<tr>
<td>P(-14)**</td>
<td>0.025</td>
<td>0.052</td>
<td>0.493</td>
<td>0.418</td>
</tr>
</tbody>
</table>

* Probability of the difference between means in the column being due to chance alone, as measured by Student’s t-test.
** Excluding K14.

7.3.4 Development of the passage of markers in koalas

The passage of digesta markers was slower in the 9 month old juveniles than in the older koalas ($k_{9} < k_{11-21}$, $P=0.01$; $k_{9} < k_{11-21}$, $P<0.000$) (Table 7.3). However, from 11 months there were no significant changes in digesta marker passage rates with either age or mass (Figure 7.2). There was no difference in the passage rate of digesta markers between juveniles from 11 months of age and adult females ($k_{9}$, $P=0.47$; $k_{11-21}$, $P=0.84$).
Figure 7.2 Development of digesta turnover time in koalas older than 250 days by a) age and b) mass.
Table 7.3 Passage of digesta markers in juvenile koalas (means, SD in brackets).

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>N</th>
<th>$k_{Co}$ (h⁻¹)</th>
<th>$k_{Cr}$ (h⁻¹)</th>
<th>$1/k_{Co}$ (h)</th>
<th>$1/k_{Cr}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>3</td>
<td>0.0041 (0.0019)</td>
<td>0.0207 (0.0019)</td>
<td>306 (198)</td>
<td>48 (4)</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>0.0118 (0.0017)</td>
<td>0.0536 (0.0142)</td>
<td>86 (12)</td>
<td>20 (6)</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>0.0113 (0.0013)</td>
<td>0.0532 (0.0067)</td>
<td>89 (10)</td>
<td>19 (2)</td>
</tr>
<tr>
<td>21</td>
<td>6</td>
<td>0.0122 (0.0033)</td>
<td>0.0396 (0.0114)</td>
<td>87 (21)</td>
<td>27 (7)</td>
</tr>
<tr>
<td>Total (without 9 months)</td>
<td>17</td>
<td>0.0118 (0.0022)</td>
<td>0.0486 (0.0127)</td>
<td>87 (14)</td>
<td>22 (7)</td>
</tr>
</tbody>
</table>

7.3.5 Estimate of MRT of digesta markers in koalas

The average transit time (TT) was 11.8 ± 3.8 h (SD, N = 18). The average values of $k_{Co}$ and $k_{Cr}$ were 0.01152 ± 0.0025 and 0.0498 ± 0.0146 respectively. Using Equation 7.6,

$$MRT = TT + 1/k$$

the MRT's of Co and Cr were 99 and 32 hours respectively.

7.4 DISCUSSION

7.4.1 Digesta Markers

Ideally, a digesta marker must:

a) not be absorbed from the gut;
b) not be affected by or affect digestive processes;
c) be intimately associated with the digesta phase which it is intended to mark;
d) be easily recovered and quantified (Koth and Luckey, 1972; Faichney, 1975).

Non-ideal behaviour of digesta markers can affect the measured passage rate of digesta, especially if the marker does not label the phase of digesta originally intended to be labelled (Cork and Warner, 1983; Foley and Hume, 1987a).
Mordanted Cr-CWC particles are stable and largely indigestible, with negligible absorption of Cr from the gut and almost complete recovery of Cr (99.5-99.9 %) (Uden et al., 1980; Foley and Hume, 1987a). However, the Cr attached to the CWC probably affects the specific gravity of the particles (Uden et al., 1980), which may affect the behaviour of particles in the digestive tract (Warner, 1981).

Cork and Warner (1983) showed that Cr-EDTA was suitable as a fluid digesta marker in koalas; over 93 % of faecal Cr was associated with fluid and only 1 % of the dose was absorbed and excreted in the urine. More Co-EDTA is absorbed from the gut than Cr-EDTA, although usually only 2-3 % is excreted in urine (Uden et al., 1980). An exception is a report of 28 % urinary excretion of Co-EDTA in rabbits (Uden et al., 1980); these authors recommended caution in the use of Co-EDTA in animals with prolonged retention of fluid digesta. Cr-EDTA is also partially adsorbed onto particulate matter (Warner, 1969), so Co-EDTA may behave similarly. Because of the large amount of small particles in the gut of the koala (Cork and Warner, 1983), adsorption of Co-EDTA onto particles would tend to include some small particles in the phase measured by this marker.

Thus, Cr-CWC and Co-EDTA are good digesta markers, with relatively small deviations from ideal behaviour. However, as the use of Co-EDTA and Cr-CWC have never been validated in koalas, the magnitude of their deviation from ideality is unknown.

7.4.2 Excretion of digesta markers in koalas

The best single measure of the rate of passage of digesta is the mean retention time (MRT) calculated from complete collection of faecally excreted marker (Warner, 1981). Total collection of faeces from free-living koalas was virtually impossible, so MRT could not be calculated directly in this study. However, it was possible to fit an exponential decay function to most of the data for individual koalas and thus estimate MRT in the major mixing compartment (Brandt and Thacker, 1958) and the total gastrointestinal tract (Sakaguchi et al., 1987).

The latter method for calculation of MRT assumes first order kinetics of digesta flow and only two components of digesta retention; the first, TT, is the time for digesta transit through all the simple portions of the gut, assuming non-mixed "plug flow", and the second is the turnover in one mixing compartment, assuming that all the dose was delivered to it as a bolus by the "plug flow" sections (Penry and Jumars, 1987; Hume, 1989). There are at least two mixing chambers in the koala gut; stomach and caecum/proximal colon (Cork and Warner, 1983), suggesting that the first order model may not accurately describe the system. However, Cork and Warner (1983) compared MRT calculated with a first order kinetic model with MRT calculated using
a direct non-compartmental method. On average the first order model underestimated the MRT of particles by only 3% and that of fluid by 2%, so the influence of the gastric component is likely to be insignificant compared with that of the hindgut. Thus the use of a first order kinetic model in this study should give a realistic approximation of overall MRT, and provide a good basis for comparison within the study.

The second phase of the Cr marker excretion represented only a very small quantity of the marker dose, as concentrations were only 1-2% of the peak concentration. A similar result was reported by Foley and Hume (1987a) using a similar sized Cr-CWC mordant in greater gliders. They suggested that the second pool of Cr marker originated from small particles in the dose due to imperfect sieving during marker preparation. It is also likely that the second phase of Cr excretion observed in this study is due to small Cr-CWC particles moving with the fluid pool, as there was no significant difference in the paired comparison of $k_{Cr2}$ and $k_{Co}$. The presence of small Cr-CWC particles could be due to imperfect sieving during marker preparation or the mastication of particles at dosing. Of these two possibilities the former is the more likely.

### Table 7.4 Digesta Retention in marsupial arboreal folivores.

<table>
<thead>
<tr>
<th>Species</th>
<th>MRT (h)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluid</td>
<td>Particles</td>
</tr>
<tr>
<td>Koala</td>
<td>99 (Co-EDTA)</td>
<td>32 (Cr-CWC)</td>
</tr>
<tr>
<td></td>
<td>213 (Cr-EDTA)</td>
<td>100 (Ru-P)</td>
</tr>
<tr>
<td>Greater glider</td>
<td>50 (Cr-EDTA)</td>
<td>23 (Cr-CWC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>46 (Ru-P)</td>
</tr>
<tr>
<td>Ringtail possum</td>
<td>65 (Cr-EDTA)</td>
<td>35 (Ru-P)</td>
</tr>
<tr>
<td>Brushtail possum</td>
<td>51 (Cr-EDTA)</td>
<td>49 (Ru-P)</td>
</tr>
<tr>
<td></td>
<td>64 (Cr-EDTA)</td>
<td>71 (Ru-P)</td>
</tr>
</tbody>
</table>

1 This study        4 Chilcott and Hume (1985)
3 Foley and Hume (1987a)

The MRT of both fluid and particles in free-living koalas in this study was considerably less than that measured in captive koalas by Cork and Warner (1983) (Table 7.4). There are several possible reasons for the large differences. First, the
MRT of Cr-CWC particles (600-1180 μm) in this study accurately reflected the passage of particles of that size through the digestive tract of the koalas. In contrast, the digesta particle marker that Cork and Warner (1983) used (Ru-P) probably overestimated particle retention; adsorption of Ru-P to the surface of particles is not irreversible, so the marker is in equilibrium with the available binding sites in the digesta particle pool which contains a far greater surface area of fine than of large particles (Faichney and Griffiths, 1978). Cork and Warner (1983) showed that within 3 hours the Ru-P marker was largely associated with fine particles (< 109 μm), and was almost exclusively so after 24 h. Thus their estimate of the MRT of particles probably includes some rapidly passed large particles, but predominately fine particles selectively retained in the hindgut (Cork and Warner, 1983). A similar situation was reported by Foley and Hume (1987a), who found the retention of Ru-P to be the same as Cr-EDTA in greater gliders and much longer than the retention of a Cr-CWC mordant. The large difference in MRT between large and small particles supports the hypothesis that large particles are excreted rapidly by the koala, reducing the "gut-filling" effect of the least digestible portion of their diet (Cork and Warner, 1983).

The second possible reason for the lower MRT's in this study lies in the greater dry matter intake of the free-ranging koalas; free-living lactating females ate 69 % more than did captive koalas (Cork et al., 1983), and non-lactating females 35 % more. Even though intake had no significant effect on fluid retention in this study, the differences in diet and activity between free-living koalas and the captive koalas of Cork and Warner (1983) may account for the difference in fluid marker retention (Warner, 1981), and probably also part of the difference in particle marker retention (Table 7.4).

There was little change in digesta passage with increasing age and mass in the juvenile koalas after 9 months. Empirical and theoretical interspecific comparisons show that MRT is expected to increase with increasing body mass as a power function with an exponent close to 0.25 (Demment and Van Soest, 1985; Ilius and Gordon, 1992), although Duncan et al. (1990) found no significant relationship of mass and MRT in a range of large herbivores.

\[
MRT = 9.4 M^{0.26} \quad \text{(Hindgut fermenters)} \quad \text{Equation 2.1}
\]

This is the result of interspecific relationships of gut capacity and energetic requirements to body mass. Gut capacity increases isometrically with body mass (Parra, 1978; Demment and Van Soest, 1985; Ilius and Gordon, 1992), whereas energy requirements, and consequently the required energy intake, increase
allometrically, with an exponent close to 0.75 (Kleiber, 1975; Nagy, 1987). However, in juvenile koalas from 400-5500 g, energetic requirements increase isometrically with mass.

\[
\text{FMR (kJ.d}^{-1}) = 0.249 \times M (g) + 149.1 \quad \text{Equation 5.2}
\]

Therefore as

\[
\text{MRT} = \frac{\text{pool size}}{\text{intake}} \quad \text{Equation 7.1}
\]

the MRT of koalas should be independent of body mass.

The long retention of both fluid and particle markers in the 9 month old juveniles was probably due to their milk diet; at that stage they were taking in very little foliage (Chapter 5). Dietary fibre stimulates gut motility (Warner, 1981), so that on the low fibre milk diet gut motility may have been low, with a corresponding high MRT. This would be especially true of the fluid marker; passage of the fibrous particle marker was significantly faster. The highly digestible nature of the milk diet (about 94 % digestible, Walker and Vickery, 1989) would have resulted in little faecal production, contributing to long marker retention. Greater gut fill, motility and faecal production in 11 month and older koalas consuming foliage explains the shorter retention in these animals than in the purely milk fed 9 month old young.

### 7.4.3 Lactation and digestive function in koalas

Lactating koalas showed a strong tendency toward faster passage of the particle marker, a smaller pool of large particles in the digesta, and a larger pool of fluid marker, while the passage of fluid was unaffected. This pattern is consistent with the limited options available to the koala.

Faster passage and a smaller pool of large particles together with a 30-40 % increase in the fluid capacity of the major mixing compartment would compensate for the gut filling effect of the 22 % greater intake due to reproductive demands, without increasing the passage of fluid and fine particles. This would minimize the loss of bacterial nitrogen (Sperber, 1968; Cork and Warner, 1983) and the decrease in digestibility associated with increases in passage rate (Blaxter et al., 1956; Grovum and Williams, 1973a; Warner, 1981).

Although the relationship of passage of the fluid digesta marker with that of small particles is unknown in koalas, Sakaguchi and Hume (1990) showed that in ringtail possums a fine particle marker moved with the fluid marker. Thus it is likely that fine
particles also move with the fluid fraction in koalas. Further evidence for particles moving with fluid comes from the second component of the Cr excretion curves in this study and that of Foley and Hume (1987a), and the long retention of the Ru-P particle marker used by Cork and Warner (1983).

Foley and Cork (1992) reviewed studies of small herbivores showing increases in gut capacity and absorptive ability with increases in intake. They concluded that such changes were probably important in meeting reproductive requirements in small herbivores. The digestive strategy of koalas to meet their reproductive requirements appears to be two-fold. First, there is an increase in the passage rate of large particles, which reduces the "gut-filling" effect of this poorly digested food fraction. Second, there is an increase in the size of the fluid pool, which allows intake to increase without the detrimental effects associated with a decrease in MRT. These digestive strategies of koalas for meeting reproductive requirements are worth further study, using larger sample sizes of both reproductive and non-reproductive koalas.
CHAPTER EIGHT

COMPOSITION OF THE DIET OF FREE-LIVING KOALAS

8.1 INTRODUCTION

"its food consists solely of gum leaves, in the choice of which it is excessively nice."

Sydney Gazette 1803 (cited in Iredale and Whitely, 1934)

The dietary selectivity of koalas has long been noted (see quote above), and in the last 15 years a number of studies have attempted to determine the basis of that selectivity. Ullrey et al. (1981) found that captive koalas preferred \textit{Eucalyptus} foliage higher in nitrogen (and hence crude protein), phosphorus and potassium and lower in ether extract (containing the essential oils and waxes), fibre, gross energy, calcium, iron and selenium than less preferred foliage. Southwell (1978) found no correlation in leaf preferences with essential oil yield or composition, though Betts (1978) reported a negative correlation between preference and the ratio of sesquiterpenoids (15-carbon compounds) to cineole (often the most abundant of the 10-carbon compounds) in the essential oil.

The toxicity or deterrent value of allelochemicals depends on the balance of nutrient gain from the food and cost associated with the allelochemicals (Janzen, 1978). Thus, more recent attempts to explain the food preferences and requirements of arboreal folivores have focussed on both macronutrients such as protein, water and fibre, and on the potentially detrimental allelochemicals such as phenolics, essential oils and alkaloids (Milton, 1979; Oates et al., 1980; McKey et al., 1981; Cork and Pahl, 1984; Oates et al., 1990; Cork, 1992; Hume and Esson, 1993). Hume and Esson (1993) found that the preferred \textit{Eucalyptus} foliage of captive koalas was lower in condensed tannins and the sesquiterpenoid fraction of essential oils, and higher in volatile monoterpenes and the ratios of nitrogen to condensed tannins and fibre. They also found that there were threshold levels of approximately 55 % and 2 % for water and essential oil content respectively, below which koalas did not accept foliage, but above which there was little relation between preference and the contents of the two components. Similarly, Pahl and Hume (1990) found threshold levels of approximately 60 % and 1.5 % of water and nitrogen content respectively for the acceptance of foliage by captive koalas. Hume and Esson (1993) also postulated that the volatile terpenoids acted as a positive feeding cue to koalas, despite the metabolic
requirements for detoxification of these allelochemicals (Cork et al., 1983; Foley and Hume, 1987; Foley, 1992).

Braithwaite et al. (1983) found that most of the arboreal marsupials in the Eucalyptus forest of south-eastern Australia were concentrated in patches of "high quality" forest. The foliage in these "high quality" patches had high values of a nutritional index largely correlated with concentrations of nitrogen, phosphorus and potassium. Similarly, Cork (1992) found that levels of nitrogen, phosphorus, and potassium, as well as ratios of nitrogen and phosphorus to measures of phenolics and fibre, were greater in Eucalyptus communities favoured by arboreal foliviore than those with few foliviore. The foliar nutrient levels and folivore density reported by Braithwaite et al. (1983) correlated positively with soil nutrient levels (Braithwaite et al., 1984), and Cork (1992) postulated that nutrient availability (sensu Resource availability hypothesis, Bryant et al., 1985a and b; Coley et al., 1985) to the trees may be the major determinant of levels of defensive compounds in Eucalyptus foliage, and hence the nutritional quality of that foliage. The broad-scale distribution of koalas in NSW and the findings of Braithwaite et al. (1983) support this hypothesis; 76% of koala sightings reported in a 1986-7 survey were on land with relatively high agricultural productivity (Reed et al., 1990).

In common with other marsupial foliviore, koalas prefer young foliage when it is available (Ullrey et al., 1981; Cork and Pahl, 1984; Landsberg, 1987; Hindell, 1984; Kavanagh and Lambert, 1990; Pahl and Hume, 1990). Young Eucalyptus foliage is generally higher than mature foliage in nitrogen, water and phenolics, and lower in fibre (Cork, 1984; Cork and Pahl, 1984; Kavanagh and Lambert, 1990; Hume and Esson, 1993). However, as young foliage represents a much less abundant or reliable resource than mature foliage, the quality of mature foliage is likely to limit the long-term persistence of foliviore populations (Cork, 1992).

Koalas are selective at the level of Eucalyptus species both within and between sites (Eberhard, 1978; Robbins and Russell, 1978; Hindell et al., 1985; Martin, 1985a; Hindell and Lee, 1987, 1988; Gordon et al., 1988, 1990; White and Kunst, 1990). They also prefer certain individual trees within species (Hindell et al., 1985; Hindell and Lee, 1987), and appear to choose trees at least partly on the basis of physical characteristics such as size and structure (Robbins and Russell, 1978; Hindell and Lee, 1987).

Lactating koalas have greater nutrient requirements than non-lactataters. These requirements are met largely by increases in food intake (Chapter 6), but could also be partially met by selection of higher quality foliage of greater metabolizable nutrient
content than that consumed by non-lactating koalas. This chapter investigates diet in free-living koalas, and compares:

1. The foliage selected by koalas with the foliage available.

2. The foliage selected by lactating koalas with that selected by non-lactating females.

8.2 MATERIALS AND METHODS

8.2.1 Collection of foliage

Samples of foliage (100-200 g fresh weight) were taken from the trees in which the koalas were found at each capture during 1991. The samples were taken from the canopy close to the koala and from where it had been feeding if possible. Foliage from trees occupied during the day is generally consumed by koalas (Robbins and Russell, 1978; Hindell et al., 1985). Although it was often not possible to confirm that the koalas were eating foliage from the trees they were captured in, or from the part of the canopy sampled, collected samples were considered to be koala food items.

The leaves were stripped from the branches and divided into two leaf age classes: mature leaves (fully expanded and deep green); and "tips" (incompletely expanded, soft, light green to reddish, often including the terminal portion of the stem). The stripped leaves were immediately divided into three subsamples. One (~10 g) was placed into a preweighed paper bag, and then reweighed. This sample was later oven-dried at 45°C for 7 days to determine dry matter content, with correction for the loss of water from the paper bag. The second subsample (20-50 g) was sealed in a plastic bag and immediately placed on ice, frozen on return to camp in the evening and then stored at -20°C until analysis for fibre, nitrogen and phenolics. The third subsample (up to 100 g) was stored in a paper bag and allowed to air-dry for later analysis of essential oil content.

8.2.2 Chemical analysis.

The frozen foliage subsamples were freeze-dried and then ground in a rotary mill (Cyclotec, Tecator). Between the nitrogen and fibre analyses the ground samples were stored in scintillation vials in a dessicator. The residual dry matter content of the ground samples was determined by oven-drying at 105°C for 24 hours at the time of analysis.
Nitrogen content was determined by the semi-micro Kjeldahl technique of Ivan et al. (1974), using a selenium catalyst. Excessive foaming during the early part of the acid digestion was minimized by raising the temperature of the digest slowly to 420°C over about 3 hours, then completing the digestion at this temperature. Recovery of nitrogen was tested by adding nicotinamide standard and found to be 99-102 %.

Phenolics were extracted from the ground foliage by an adaptation of the method of Cork and Krockenberger (1990). Ground foliage (250 mg) was weighed into a centrifuge tube and then 6 ml acetone (50 % v/v in water) added. The tubes were capped and mixed by inversion, then extracted by continuous mixing on a vertical turn-table at 5°C in the dark for 30 min. The tubes were then centrifuged at 3000 rpm for 10 min., the supernatant collected, and the extraction step repeated three more times. The volume of acetone (50 %) used in the final extraction was only 4 ml, making a total volume of 22 ml.

The collected supernatant was bulked in a scintillation vial and stored at -20°C. An aliquot (2 ml) of the supernatant was freeze-dried, then made back up to volume with water for analysis of protein-precipitating capacity and phenolic content. Phenolic content was determined by the method of Hagerman and Butler (1978), as modified by Cork (1992), and protein-precipitating capacity determined by the method of Hagerman and Robbins (1987). Protein-precipitating capacity is henceforth referred to as PP (mg.g⁻¹ dry foliage), and the total phenolic content as TP (mg.g⁻¹ dry foliage). The residue was stored at -20°C until analysis for fibre content.

The phenolic-free residue was analysed for neutral-detergent fibre (NDF) by the method of Goering and Van Soest (1970), with the omission of sodium sulphite (Cork, 1984; Van Soest et al., 1991).

The total essential oil content of the foliage was determined by steam-distillation of the air-dried subsample (Hughes, 1970) and the components of the oil separated by gas-liquid chromatography (GC). The oil (0.5 μl) was injected onto the capillary column (DB-wax, 30 m x 0.25 mm) of a Varian 3400 Gas Chromatograph and detected with a FID detector. Nitrogen was used as the carrier gas, the injector held at 250°C, split ratio 1:50, column from 50-250°C, and the detector at 300°C. The GC traces were recorded onto an IBM compatible computer using the DATACAN data acquisition program, integrated and divided into four 14-minute segments. The proportion of the oil eluted in each segment was expressed as a percentage of the total. As elution time is highly correlated with molecular weight, the early fractions (Oil₁₄ and Oil₂₈) represented the more volatile, low molecular weight terpenoids, and
the later fractions (Oil$_{42}$ and Oil$_{56}$) the less volatile, waxy, high molecular weight sesquiterpenoids (Betts, 1978).

8.2.3 Tree species

Overall tree-species usage was determined from all (1,010) day-time observations of koalas. The presence of any new foliage (tips) was recorded. The relative abundance of each tree species was measured by counting all trees on the study grid with a diameter at breast height of greater than 100 mm; trees under this diameter were rarely used by koalas.

The use of tree species was compared between lactating and non-lactating females using all sightings between the months of May and December (inclusive) in both years of the study for which the reproductive status of the female was known. This period was selected because it covered the time of greatest lactational output (Chapter 5).

8.2.4 Selection and collection of "Control" foliage

In July 1991 and January 1992 foliage samples were collected from trees not known to have been used by koalas. These trees were chosen by randomly selecting 15 of the 100 one-hectare grid squares and then randomly selecting one tree of each species present on the grid square. These "control" samples were analysed as described above. Because the method of selection ensured that trees were chosen randomly within each species, but not between species, proportions of species among the control trees were biased toward less abundant species, especially E. stellulata, which made up 24 % of control trees but only 6.3 % of the total trees (Table 8.3). To avoid bias from this source, the proportions of each species among the control trees were set at that measured for the entire site (Table 8.3) for comparisons of the chemical composition of foliage from trees utilized by koalas with that generally available (i.e. from control trees).

8.2.5 Statistical.

The proportion of utilization of the available tree species was compared with the measured species composition of the site by a "G" goodness-of-fit test (Sokal and Rohlf, 1981). Tree species use of lactating and non-lactating females during the months of May to December were separately compared with the species use by all females during the same period ("G" goodness-of-fit test).
Multivariate analyses of the relationship of foliar chemical composition and consumption by koalas were not performed due to significant correlations (Spearman's coefficient of rank correlation, Wilkinson, 1989) among components (Table 8.1). Instead, differences among means were tested by nonparametric tests, as variances were found by Bartlett's test (Wilkinson, 1989) to be heterogeneous in almost all cases. The Kruskal-Wallis k-sample test and Mann-Whitney U test were used to test for differences between means (Wilkinson, 1989).

The composition of tips was compared with that of mature foliage from the same tree for January 1992 as most tip samples were from this time, using the Wilcoxon signed rank test for paired samples (Wilkinson, 1989). All other comparisons were made on mature foliage.

The composition of foliage from the four major *Eucalyptus* species on the site was compared in July 1991 and January 1992 using "control" foliage collected at those times.
The foliar constituents are abbreviated as follows: DM = dry matter (% w/w), N = nitrogen (% w/w), NDF = neutral detergent fibre (% w/w), PP = protein-precipitating capacity (mg.g⁻¹), TP = total phenolics (mg.g⁻¹), Oil = essential oil content (% v/w), Oil₁₄ = the proportion of the oil eluted from the GC column between 0 and 14 minutes, Oil₂₈ = the proportion of oil eluted from 14-28 min., and similarly for Oil₄₂ and Oil₅₆.

Table 8.1 Correlation matrix of foliage chemical composition from the January 1992 measurement period (Spearman's correlation coefficients, n=55)

|         | Oil₁₄ | Oil₂₈ | Oil₄₂ | Oil₅₆ | Oil₁₄² | Oil₂₈² | Oil₄₂² | Oil₅₆² | DMT² |Oil₁₄Oil₂₈ | Oil₁₄Oil₄₂ | Oil₁₄Oil₅₆ | Oil₂₈Oil₄₂ | Oil₂₈Oil₅₆ | Oil₄₂Oil₅₆ | Oil₁₄DMT | Oil₂₈DMT | Oil₄₂DMT | Oil₅₆DMT |
|---------|-------|-------|-------|-------|---------|---------|---------|---------|-------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| DM      | 0.86  | 0.68  | 0.62  | 0.60  | 0.57   | 0.51   | 0.48   | 0.44   | 0.33 | 0.23     | 0.25     | 0.27     | 0.25     | 0.23     | 0.27     | 0.25     | 0.23     |
| Oil₁₄   |       |       |       |       |        |        |        |        |      |          |          |          |          |          |          |          |          |          |
| Oil₂₈   |       |       |       |       |        |        |        |        |      |          |          |          |          |          |          |          |          |          |
| Oil₄₂   |       |       |       |       |        |        |        |        |      |          |          |          |          |          |          |          |          |          |
| Oil₅₆   |       |       |       |       |        |        |        |        |      |          |          |          |          |          |          |          |          |          |
| DMT     |       |       |       |       |        |        |        |        |      |          |          |          |          |          |          |          |          |          |
8.3 RESULTS

8.3.1 Availability and composition of tips

The availability of tips varied seasonally (Figure 8.1), being lowest in winter and early spring and highest in summer. Tips were less available in 1991 than 1990; no trees used by koalas during August-October 1991 had tips, in contrast to the same period in 1990, when around 50% of trees had tips (Figure 8.1). This corresponds with the low rainfall during the winter and spring of 1991 (Chapter 6).

![Figure 8.1 The proportion of trees used by koalas, which had new foliage.](image)

The composition of tips differed from that of mature foliage; tips had more nitrogen, higher ratios of nitrogen to fibre and phenolics, greater water content, less of the defensive fibre and oil components, but more phenolics (Table 8.2). There was also a trend toward a higher ratio of nitrogen to the protein-precipitating capacity of the tips than in the mature foliage (Mean difference = 0.6 ± 2.0, P = 0.063).
Table 8.2 Composition of tips and mature foliage (dry matter basis).

<table>
<thead>
<tr>
<th>Component</th>
<th>N</th>
<th>Tip (mean ± SD)</th>
<th>Mature (mean ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>32</td>
<td>29.4 ± 6.0</td>
<td>45.8 ± 6.2</td>
<td>***</td>
</tr>
<tr>
<td>(%) fresh weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>47</td>
<td>2.26 ± 0.42</td>
<td>1.60 ± 0.23</td>
<td>*</td>
</tr>
<tr>
<td>Fibre (NDF) (%)</td>
<td>45</td>
<td>31.5 ± 5.4</td>
<td>35.2 ± 4.6</td>
<td>***</td>
</tr>
<tr>
<td>N: NDF (g/g)</td>
<td>45</td>
<td>0.075 ± 0.024</td>
<td>0.046 ± 0.010</td>
<td>***</td>
</tr>
<tr>
<td>TP (mg.g⁻¹)</td>
<td>46</td>
<td>257 ± 145</td>
<td>234 ± 87</td>
<td>*</td>
</tr>
<tr>
<td>N: TP (g/g)</td>
<td>46</td>
<td>0.133 ± 0.104</td>
<td>0.079 ± 0.035</td>
<td>***</td>
</tr>
<tr>
<td>N: PP(g/g)</td>
<td>46</td>
<td>0.145 ± 0.300</td>
<td>0.053 ± 0.035</td>
<td>***</td>
</tr>
<tr>
<td>Oil (% v/w)</td>
<td>10</td>
<td>2.9 ± 2.0</td>
<td>3.0 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>Oil2₈ (%)</td>
<td>10</td>
<td>24.5 ± 15.4</td>
<td>21.7 ± 14.2</td>
<td>*</td>
</tr>
</tbody>
</table>

The foliar constituents are abbreviated as follows: NDF = neutral detergent fibre (% w/w), PP = protein-precipitating capacity (mg.g⁻¹), TP = total phenolics (mg.g⁻¹), Oil = essential oil content (% v/w), Oil₂₈ = the proportion of oil eluted from the GC column between 14 and 28 min.

8.3.2 Utilization of available tree species

Koalas used tree species in different proportions to those in which they were present on the site (G= 680, 5 DF, P< 0.005). *E. acaciiformis* and *E. viminalis* were used more than would be expected, *E. radiata* was used about as often as expected, and *E. pauciflora, E. stellulata, Acacia falciformis, A. melanoxylon, Banksia integrifolia* and *Allocasuarina littoralis* were used less than expected from their occurrence (Table 8.3). *E. acaciiformis* was selected most strongly (48 % of observations but only 17 % of relative abundance), and *E. pauciflora* was least selected (only 32 % of those utilized but 50 % of the available trees).

There were 8705 trees over 100 mm dbh on the study site, but only 796 (9.1 %) individual trees were observed to be used by koalas during the study period. Koalas used 19 % of the *E. acaciiformis* trees and only 7 % of the *E. pauciflora* trees.

Neither lactating nor non-lactating females used tree species in significantly different proportions to those observed for females in general (L G_adj=3.66, 0.5<P<0.75; NL G_adj=6.75, 0.1<P<0.25), although lactaters tended to use *E. acaciiformis* more and *E. radiata* less than did non-lactaters (Table 8.4).
Table 8.3 Utilization of tree species by koalas.

<table>
<thead>
<tr>
<th>Species</th>
<th>Abundance</th>
<th></th>
<th>Utilized</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% of total</td>
<td>No.</td>
<td>% of total</td>
</tr>
<tr>
<td>E. acaciiformis</td>
<td>1507</td>
<td>17</td>
<td>485</td>
<td>48</td>
</tr>
<tr>
<td>E. pauciflora</td>
<td>4381</td>
<td>50</td>
<td>323</td>
<td>32</td>
</tr>
<tr>
<td>E. radiata</td>
<td>1401</td>
<td>16</td>
<td>142</td>
<td>14</td>
</tr>
<tr>
<td>E. stellulata</td>
<td>546</td>
<td>6.3</td>
<td>25</td>
<td>2.5</td>
</tr>
<tr>
<td>E. viminalis</td>
<td>12</td>
<td>0.1</td>
<td>22</td>
<td>2.2</td>
</tr>
<tr>
<td>A. falciformis</td>
<td>11</td>
<td>0.1</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>A. melanoxylon</td>
<td>161</td>
<td>1.8</td>
<td>3</td>
<td>0.3</td>
</tr>
<tr>
<td>B. integrifolia</td>
<td>137</td>
<td>1.6</td>
<td>3</td>
<td>0.3</td>
</tr>
<tr>
<td>Allocasuarina littoralis</td>
<td>549</td>
<td>6.3</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>8705</strong></td>
<td><strong>100</strong></td>
<td><strong>1010</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 8.4 Use of tree species by female koalas in the period May-December.

<table>
<thead>
<tr>
<th>Species</th>
<th>Lactating</th>
<th></th>
<th>Non-lactating</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% of total</td>
<td>No.</td>
<td>% of total</td>
</tr>
<tr>
<td>E. acaciiformis</td>
<td>113</td>
<td>47.3</td>
<td>40</td>
<td>38.1</td>
</tr>
<tr>
<td>E. pauciflora</td>
<td>73</td>
<td>30.5</td>
<td>26</td>
<td>24.8</td>
</tr>
<tr>
<td>E. radiata</td>
<td>36</td>
<td>15.1</td>
<td>22</td>
<td>21.0</td>
</tr>
<tr>
<td>E. stellulata</td>
<td>7</td>
<td>2.9</td>
<td>4</td>
<td>3.8</td>
</tr>
<tr>
<td>E. viminalis</td>
<td>3</td>
<td>1.3</td>
<td>6</td>
<td>5.7</td>
</tr>
<tr>
<td>E. saligna</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1.9</td>
</tr>
<tr>
<td>A. falciformis</td>
<td>3</td>
<td>1.3</td>
<td>4</td>
<td>3.8</td>
</tr>
<tr>
<td>A. melanoxylon</td>
<td>2</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B. integrifolia</td>
<td>1</td>
<td>0.4</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>Allocasuarina littoralis</td>
<td>1</td>
<td>0.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>239</strong></td>
<td><strong>100</strong></td>
<td><strong>105</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

NB. E. saligna was not present on the study grid. The record of its use by a koala came from an animal that left the study site.
8.3.3 Composition of the foliage of the major *Eucalyptus* species present on the study site

There were significant differences among the four species of *Eucalyptus* present on the study site (control trees only) in almost every foliar constituent measured in both winter (July) and summer (January) (summary Table 8.5; mean values Table 8.6). The patterns were similar in summer and winter.

### Table 8.5 Comparison of the foliar composition of four species of *Eucalyptus*¹.

<table>
<thead>
<tr>
<th>Component</th>
<th>( P_{\text{winter}} )</th>
<th>Rank</th>
<th>( P_{\text{summer}} )</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM²</td>
<td>&lt;0.001</td>
<td>SAR</td>
<td>0.110</td>
<td>NS</td>
</tr>
<tr>
<td>N</td>
<td>0.007</td>
<td>SRA</td>
<td>0.106</td>
<td>NS</td>
</tr>
<tr>
<td>NDF</td>
<td>0.016</td>
<td>ARP</td>
<td>0.001</td>
<td>ARA</td>
</tr>
<tr>
<td>TP</td>
<td>&lt;0.001</td>
<td>PASR</td>
<td>&lt;0.001</td>
<td>PRAS</td>
</tr>
<tr>
<td>PP</td>
<td>&lt;0.001</td>
<td>PAR</td>
<td>&lt;0.001</td>
<td>PRAS</td>
</tr>
<tr>
<td>N:NDF</td>
<td>0.001</td>
<td>NS</td>
<td>&lt;0.014</td>
<td>SRA</td>
</tr>
<tr>
<td>N:TP</td>
<td>&lt;0.001</td>
<td>SRA</td>
<td>&lt;0.001</td>
<td>SRA</td>
</tr>
<tr>
<td>N:PP</td>
<td>&lt;0.001</td>
<td>SRA</td>
<td>0.002</td>
<td>SRA</td>
</tr>
<tr>
<td>Oil</td>
<td>&lt;0.001</td>
<td>SPR</td>
<td>&lt;0.001</td>
<td>SRA</td>
</tr>
<tr>
<td>Oil(_{14})</td>
<td>0.021</td>
<td>PRA</td>
<td>0.086</td>
<td>SRA</td>
</tr>
<tr>
<td>Oil(_{28})</td>
<td>&lt;0.001</td>
<td>PRA</td>
<td>&lt;0.001</td>
<td>PRA</td>
</tr>
<tr>
<td>Oil(_{42})</td>
<td>&lt;0.001</td>
<td>RAS</td>
<td>&lt;0.001</td>
<td>RAS</td>
</tr>
<tr>
<td>Oil(_{46})</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>RPS</td>
</tr>
</tbody>
</table>

¹ Foliage from control trees only.
² The foliar components are: DM = dry matter (% w/w), N = nitrogen (% w/w), NDF = neutral detergent fibre (% w/w), PP = protein-precipitating capacity (mg.g\(^{-1}\)), TP = total phenolics (mg.g\(^{-1}\)), N:NDF = ratio of nitrogen to fibre (g.g\(^{-1}\)), N:PP = ratio of nitrogen to PP (g.g\(^{-1}\)), N:TP = ratio of nitrogen to TP (g.g\(^{-1}\)), Oil = essential oil content (% v/w), Oil\(_{14}\) = the proportion of the oil eluted from the GC column between 0 and 14 minutes, Oil\(_{28}\) = the proportion of oil eluted from 14-28 min., and similarly for Oil\(_{42}\) and Oil\(_{46}\).
³ The *Eucalyptus* species names are abbreviated as follows: A = *Eucalyptus acaciiformis*, P = *Eucalyptus pauciflora*, R = *Eucalyptus radiata*, S = *Eucalyptus stellulata*.
⁴ Underlining joins means in any one group. Species are listed in order of ascending value of the component from left to right. Double-underlining indicates that the mean indicated can be grouped with both the neighbouring means, but that the neighbouring means are distinct from each other.
⁵ Although the Kruskal-Wallis test indicated differences between the means, they could not be grouped by the Mann-Whitney U test at the 5 % level.
Table 8.6 Composition of the foliage from four species of *Eucalyptus* (means ± SD).

<table>
<thead>
<tr>
<th></th>
<th>EA</th>
<th>EP</th>
<th>ER</th>
<th>ES</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>45.9b± 1.4</td>
<td>48.7A± 2.5</td>
<td>46.5AB± 2.7</td>
<td>43.5C± 2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(18)</td>
<td>(6)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>46.1± 3.3</td>
<td>45.9A± 7.3</td>
<td>41.2± 9.0</td>
<td>41.6± 4.6</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(12)</td>
<td>(4)</td>
<td>(9)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>1.72b± 0.27</td>
<td>1.42± 0.14</td>
<td>1.51± 0.04</td>
<td>1.41A± 0.09</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(18)</td>
<td>(6)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>1.64A± 0.15</td>
<td>1.60± 0.37</td>
<td>1.61± 0.14</td>
<td>1.47A± 0.17</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(16)</td>
<td>(5)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>NDF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>33.1A± 9.1</td>
<td>36.4A± 2.9</td>
<td>34.6± 3.0</td>
<td>38.5± 3.2</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(18)</td>
<td>(6)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>32.2B± 4.5</td>
<td>39.1A± 4.7</td>
<td>34.0AB± 3.6</td>
<td>36.5AB± 3.9</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(15)</td>
<td>(5)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>PP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>382A± 116</td>
<td>199B± 73</td>
<td>364A± 74</td>
<td>468A± 120</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(18)</td>
<td>(6)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>470A± 177</td>
<td>244B± 78</td>
<td>382AB± 83</td>
<td>546A± 176</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(15)</td>
<td>(5)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>238± 49</td>
<td>162B± 58</td>
<td>269± 37</td>
<td>258± 36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(18)</td>
<td>(6)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>292± 90</td>
<td>147B± 31</td>
<td>267± 29</td>
<td>303± 91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(15)</td>
<td>(5)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>N:NDF</td>
<td>(x 10²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>5.5A± 1.5</td>
<td>3.9± 0.5</td>
<td>4.4± 0.3</td>
<td>3.7B± 0.4</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(18)</td>
<td>(6)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>5.2A± 1.0</td>
<td>4.1A± 1.2</td>
<td>4.8± 0.6</td>
<td>4.1A± 0.7</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(15)</td>
<td>(5)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>N:PP</td>
<td>(x 10²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>5.1B± 2.4</td>
<td>7.9A± 2.7</td>
<td>4.3± 0.9</td>
<td>4.3B± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(18)</td>
<td>(6)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>4.4B± 3.2</td>
<td>7.9± 5.7</td>
<td>4.4± 1.1</td>
<td>3.3B± 2.0</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(15)</td>
<td>(5)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>N:TP</td>
<td>(x 10²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>7.6B± 2.4</td>
<td>9.6± 2.9</td>
<td>5.7± 0.7</td>
<td>5.5B± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(18)</td>
<td>(6)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>6.5B± 3.7</td>
<td>11.2A± 3.6</td>
<td>6.1± 1.1</td>
<td>5.6B± 2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(15)</td>
<td>(5)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>Oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>1.0± 0.4</td>
<td>4.2B± 2.1</td>
<td>9.2± 0.6</td>
<td>0.5± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(18)</td>
<td>(6)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>0.7± 0.3</td>
<td>3.7± 1.2</td>
<td>9.0± 0.5</td>
<td>0.3± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(15)</td>
<td>(3)</td>
<td>(9)</td>
<td></td>
</tr>
<tr>
<td>Oil14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>54.9± 14.4</td>
<td>30.4± 13.6</td>
<td>37.8± 4.6</td>
<td>33.3± 22.8</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(16)</td>
<td>(6)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>43.5± 17.8</td>
<td>42.0± 11.9</td>
<td>43.3± 6.4</td>
<td>25.1± 16.1</td>
<td>0.086</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(15)</td>
<td>(5)</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Oil28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>19.1± 6.0</td>
<td>7.8± 4.2</td>
<td>44.6± 8.2</td>
<td>16.5± 9.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(16)</td>
<td>(6)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>27.6± 10.2</td>
<td>8.1± 1.7</td>
<td>42.6± 7.2</td>
<td>24.0± 7.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(15)</td>
<td>(5)</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Oil42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>20.8± 8.5</td>
<td>58.9± 12.5</td>
<td>15.6± 6.9</td>
<td>45.3± 19.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(16)</td>
<td>(6)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>23.4± 10.1</td>
<td>47.5± 10.9</td>
<td>11.7± 4.1</td>
<td>45.5± 18.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(15)</td>
<td>(5)</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Oil56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>5.1± 3.1</td>
<td>3.0± 0.8</td>
<td>2.0± 0.6</td>
<td>4.9± 3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(16)</td>
<td>(6)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>5.6± 2.3</td>
<td>2.4± 0.9</td>
<td>2.3± 0.6</td>
<td>5.4± 2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(15)</td>
<td>(5)</td>
<td>(8)</td>
<td></td>
</tr>
</tbody>
</table>

NB. Means bearing the same superscripts do not differ at P=0.05 (see Table 8.5). Abbreviations as for Table 8.5. Sample sizes are given in parentheses.
In summary, the most utilized species, *Eucalyptus acaciiformis*, tended to be relatively high in content of nitrogen (crude protein), in the ratios of nitrogen to NDF, PP and TP, and in the proportion of the volatile fractions of the essential oil (Oil$\text{}_{14}$ and Oil$_{28}$), but relatively low in content of fibre (NDF), dry matter (and hence high in water content), total essential oil and in a less volatile fraction of oil (Oil$_{42}$).

The least utilized species, *Eucalyptus pauciflora* and *Eucalyptus stellulata*, tended to have the opposite trends in composition to that of *Eucalyptus acaciiformis*, while *Eucalyptus radiata*, which was used in the proportion in which it occurred, tended to be more like *Eucalyptus acaciiformis* than the other two species.

8.3.4 Composition of foliage from trees utilized by koalas

8.3.4.1 Comparison of foliage from trees used by koalas with that generally available

Differences between the diet of koalas and foliage available at the Nowendoc site were largely not significant at $P=0.05$, so Table 8.7 lists significance to $P=0.10$. Thus, results indicate trends which require verification with larger samples.

In summer, levels of dry matter and the ratio of nitrogen to fibre tended to be higher in the diet of the koalas than control foliage, and levels of fibre and the third essential oil fraction (Oil$_{42}$) lower (Table 8.7). The ratio of nitrogen to fibre tended to be higher in the selected foliage than in the controls in winter, but in contrast to summer, the winter diet tended to be higher in the second and last oil fractions (Oil$_{28}$ and Oil$_{56}$) and lower in total essential oil (Table 8.7).

Koalas seem to have selected foliage on different criteria within tree species than among tree species, and between summer and winter. In summer, neither the *E. pauciflora* nor *E. radiata* foliage from trees selected by koalas were different from controls of the same species. The summer *E. acaciiformis* foliage from trees selected by koalas tended to be lower in protein-precipitating capacity and higher in the ratio of nitrogen to protein-precipitating capacity than control *E. acaciiformis* foliage (Table 8.8). In winter, foliage from *E. acaciiformis* selected by koalas was higher in dry matter, and tended to be higher in the ratio of nitrogen to total phenolics and lower in total phenolics than control *E. acaciiformis* foliage (Table 8.8). Winter foliage of *E. pauciflora* selected by koalas tended to be higher in dry matter and lower in nitrogen than control *E. pauciflora* foliage, while *E. radiata* foliage from trees selected by koalas in winter was lower in oil, and tended to lower in fibre and higher
in the ratio of nitrogen to total phenolics than that of control *E. radiata* foliage (Table 8.8).

**Table 8.7 Summary of the comparison of foliage from trees selected by koalas with the available foliage (means ± SD).**

<table>
<thead>
<tr>
<th>Component</th>
<th>Koala</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>Winter</td>
<td>47.8 ± 3.6</td>
<td>47.4 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>47.8 ± 5.6</td>
<td>44.5 ± 7.1</td>
</tr>
<tr>
<td>NDF</td>
<td>Winter</td>
<td>35.7 ± 7.1</td>
<td>36.4 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>34.1 ± 3.8</td>
<td>36.8 ± 5.3</td>
</tr>
<tr>
<td>N:NDF</td>
<td>Winter</td>
<td>0.048 ± 0.014</td>
<td>0.041 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>0.049 ± 0.009</td>
<td>0.044 ± 0.012</td>
</tr>
<tr>
<td>Oil</td>
<td>Winter</td>
<td>2.8 ± 2.6</td>
<td>4.2 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>3.7 ± 2.6</td>
<td>3.4 ± 2.5</td>
</tr>
<tr>
<td>Oil_{14}</td>
<td>Winter</td>
<td>36.3 ± 18.6</td>
<td>37.1 ± 15.8</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>45.4 ± 13.0</td>
<td>39.5 ± 13.7</td>
</tr>
<tr>
<td>Oil_{28}</td>
<td>Winter</td>
<td>25.2 ± 15.3</td>
<td>17.9 ± 15.2</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>22.7 ± 15.1</td>
<td>19.8 ± 15.6</td>
</tr>
<tr>
<td>Oil_{42}</td>
<td>Winter</td>
<td>33.5 ± 18.7</td>
<td>41.7 ± 22.8</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>28.3 ± 16.5</td>
<td>37.0 ± 18.0</td>
</tr>
<tr>
<td>Oil_{56}</td>
<td>Winter</td>
<td>5.1 ± 4.6</td>
<td>3.2 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>3.5 ± 2.6</td>
<td>3.7 ± 2.6</td>
</tr>
</tbody>
</table>

*P* is the probability of a difference between control foliage and that from trees used by koalas.

Abbreviations as in Table 8.5.
Table 8.8 Summary of differences, within a species, between foliage of trees used by koalas and that of control trees.1

<table>
<thead>
<tr>
<th>Component</th>
<th>Season</th>
<th>EA</th>
<th>P</th>
<th>EP</th>
<th>P</th>
<th>ER</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>Winter</td>
<td>K&gt;C</td>
<td>0.004</td>
<td>K&gt;C</td>
<td>0.053</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Winter</td>
<td>K&lt;C</td>
<td>0.072</td>
<td>K&lt;C</td>
<td>0.088</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDF</td>
<td>Winter</td>
<td>K&lt;C</td>
<td>0.088</td>
<td>K&lt;C</td>
<td>0.088</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td>Winter</td>
<td>K&lt;C</td>
<td>0.085</td>
<td>K&gt;C</td>
<td>0.055</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N: TP</td>
<td>Winter</td>
<td>K&gt;C</td>
<td>0.075</td>
<td>K&gt;C</td>
<td>0.055</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP</td>
<td>Summer</td>
<td>K&gt;C</td>
<td>0.099</td>
<td>K&gt;C</td>
<td>0.065</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil</td>
<td>Winter</td>
<td>K&lt;C</td>
<td>0.011</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 EA is E. acaciiformis, EP is E. pauciflora and ER is E. radiata. K stands for foliage from trees selected by koalas and C represents foliage from control trees. Other abbreviations as in Table 8.5.

8.3.4.2 Comparison between lactating and non-lactating females

There were only two statistically significant differences between the diet of lactaters and non-lactaters; in October/November the total phenolic content (TP) was lower and the ratio of nitrogen to phenolics (N:TP) was higher in the diet of lactaters (P= 0.045).

There were no differences between the chemical composition of the foliage from trees selected by adult female and juvenile koalas, based on comparisons made in August/September 1991 (21 month juveniles) and January 1992 (13 month juveniles).

The levels of measured components in the foliage from trees selected by koalas varied throughout the year (Appendix 1), especially around the peak of lactation (September/October 1991) (Table 8.9). Foliage from trees selected by koalas in September/October was higher than that in August/September in: nitrogen, the ratio of nitrogen to fibre (N:NDF), protein-precipitating capacity (PP), total phenolics (TP) and the first essential oil fraction (Oil₁₄); and lower in: fibre (NDF), the ratios of nitrogen to both protein-precipitating capacity and total phenolics (N:PP and N:TP), and the third essential oil fraction (Oil₄₂).
Table 8.9 Changes in the chemical composition of the diet of koalas during peak lactation (means ± SD)

| Component | N  | Aug/Sept 91     | Sept/Oct 91    | P1  |
|-----------|----|-----------------|----------------|
| DM²       | 12 | 52.8 ± 2.8      | 51.6 ± 2.1     | 0.388|
| N         | 13 | 1.48 ± 0.12     | < 1.74 ± 0.52  | 0.016|
| NDF       | 13 | 36.1 ± 2.2      | > 31.1 ± 2.9   | 0.003|
| PP        | 13 | 281 ± 105       | < 388 ± 89     | 0.016|
| TP        | 13 | 169 ± 51        | < 242 ± 49     | 0.003|
| N:NDF     | 13 | 0.042 ± 0.006   | < 0.058 ± 0.025| 0.005|
| N:PP      | 13 | 0.062 ± 0.021   | > 0.052 ± 0.034| 0.046|
| N:TP      | 13 | 0.099 ± 0.028   | > 0.083 ± 0.057| 0.039|
| Oil       | 12 | 3.53 ± 1.6      | 3.6 ± 1.8      | 0.392|
| Oil₁₄     | 12 | 43.6 ± 13.6     | < 57.2 ± 12.4  | 0.015|
| Oil₂₈     | 12 | 17.0 ± 10.1     | 21.4 ± 7.5     | 0.158|
| Oil₄₂     | 12 | 36.5 ± 14.8     | > 18.8 ± 10.0  | 0.008|
| Oil₆₆     | 12 | 2.8 ± 1.0       | 2.5 ± 0.9      | 0.388|

1 Probability determined from the Wilcoxon ranked sum test for paired samples
2 Abbreviations for components are as in Table 8.5.

8.4 DISCUSSION

8.4.1 Analytical methods

The foliar constituents and indices considered in this study were chosen for their ecological relevance. Nitrogen, fibre and dry matter provide estimates of the crude protein, cell wall and water content of the foliage. Lipid and energy content of foliage were not measured, as the essential oils can dominate these constituents and have a negative metabolic energy value, as they must be detoxified and excreted (Foley and Hume, 1987). This makes the significance of lipid and energy content of Eucalyptus foliage difficult to assess and of limited utility.

The two measures of phenolics were protein-precipitating capacity, which is an estimate of the activity of tannins in the foliage (Hagerman and Robbins, 1987) and total phenolics, which indicates the total defensive phenolic content and consists of condensed and hydrolysable tannins as well as non-tannin phenolics (Hagerman and Butler, 1978). The total phenolic content may be of importance in the nutritional...
quality of foliage to marsupial folivores due to its effects on acid-base balance when absorbed and excreted (Foley, 1992). The sugar moieties of hydrolysable tannins may provide metabolic energy, as hydrolysable tannins of *Eucalyptus* foliage are highly digestible, but because of the potential negative effects of the tannins the contribution of the sugar moieties is difficult to assess (Cork *et al*., 1983).

The determination of essential oil content by steam distillation (Hughes, 1970) has been criticized on the basis of introduction of artifact changes in oil composition and differing extraction efficiency of the individual components of the oil (Von Rudloff, 1975; Betts, 1978). However, essential oil composition measured after steam distillation of *Eucalyptus dives* was identical to that of oil removed directly from the oil glands (Lassak, unpublished, cited in Foley *et al*., 1987), so it is unlikely that the use of steam distillation in this study resulted in large errors in the yield and composition of essential oils. *Eucalyptus* essential oils are comprised of many components. Rather than identifying individual components, the oils were grouped into fractions on the basis of volatility in order to test the predictions of Betts (1978) and Hume and Esson (1993) about the relative composition of the essential oils in foliage selected by koalas.

### 8.4.2 Use of the available tree species

Koalas strongly preferred two of the *Eucalyptus* species present on the site, *E. acaciiformis* and *E. viminalis*, while avoiding *E. pauciflora*, and *E. stellulata*. *E. radiata* was used approximately in the proportion in which it occurred, and the non-eucalypt species were rarely used. Similar patterns of preference have been identified in other studies, though the actual species preferred or rejected vary regionally (Eberhard, 1978; Robbins and Russell, 1978; Hindell *et al*., 1985; Martin, 1985a; Hindell and Lee, 1987, 1988; Gordon *et al*., 1988, 1990; White and Kunst, 1990). Tree-species use was similar in lactating and non-lactating female koalas during the late, most energetically demanding part of lactation, though there was a trend toward lactaters using a higher proportion of *E. acaciiformis* and a lower proportion of *E. radiata* than non-lactaters.

Although physical factors such as size and shape of the trees are probably important in tree choice by koalas, especially the choice of individual trees within a species (Robbins and Russell, 1978; Hindell and Lee, 1987), differences in foliar composition among the species (Table 8.5) appear to play a major role in determining preferences of koalas, at least at this study site. The most preferred species, *E. acaciiformis*, tended to be relatively high in nitrogen, the ratios of nitrogen to fibre, phenolics and protein-precipitating capacity, and the volatile fractions of the essential oils, and
relatively low in fibre and the third oil fraction (Oil$_{42}$, largely sesquiterpenoids). The least preferred species, *E. stellulata*, was the lowest in water, nitrogen, ratios of nitrogen to fibre, phenolics, and protein-precipitating capacity, total oils and the most volatile oil fraction, and high in fibre, phenolics and protein-precipitating capacity. These constituents have variously been correlated with food choice by captive koalas in similar ways in previous studies (Ullrey *et al.*, 1981; Betts, 1978; Hume and Pahl, 1990; Hume and Esson, 1993). Hume and Esson (1993) suggested that foliage selection by keepers can bias the quality of foliage available to captive koalas and hence the patterns of preference. This study of free-living koalas was free from that bias, and the results support the earlier studies on captive koalas.

Despite its positive factors (above), the foliage of *E. acaciiformis* was high in phenolics and activity of tannins; these constituents have been correlated with rejection of foliage by folivores (Oates *et al.*, 1977, 1980; Hume and Esson, 1993). Other studies have suggested that the absolute quantity of the phenolic fraction or its activity does not in itself act as a feeding inhibitor, but that the ratio of primary nutrients to negative factors in the leaf (such as phenolics and fibre) may be more important (McKey *et al.*, 1981; Oates *et al.*, 1990; Cork, 1992). Thus it is likely that the high levels of phenolics and protein-precipitating capacity in the foliage of *E. acaciiformis* compared with the other species considered do not correlate with rejection of that species by koalas because the ratios of nitrogen to fibre, phenolics and protein-precipitating capacity are relatively high. In this respect it is interesting that the foliage of the rejected species (*E. pauciflora*) is also high in the ratios of nitrogen to phenolics and protein-precipitating capacity, but is low in nitrogen and high in fibre and the less volatile oil fraction (Oil$_{42}$). Clearly the preference of koalas for *Eucalyptus* species is not correlated with only one constituent, but with some net effect resulting from combined positive and negative effects of several constituents.

Alternatively, the preference of koalas at the Nowendoc site for *E. acaciiformis* could merely reflect their distribution within the site; indeed the highest densities of koalas coincided with high densities of *E. acaciiformis*. However, this explanation is unlikely for two reasons. First, the scale of heterogeneity in tree species distribution is small enough to ensure that all koalas have access to both *E. acaciiformis* and *E. pauciflora* within their home ranges; the distances between clumps of *E. acaciiformis* and *E. pauciflora* are within the range of one day's movement. Second, differences in foliar composition between the species, and correlations of those differences with preference by the koalas, fit well with predictions from studies of the food choice of captive koalas, making it reasonable to suggest that koalas prefer the foliage of *E. acaciiformis* on the basis of nutritional factors. For these reasons I have not
considered the species preferences of individual koalas (as in Hindell and Lee, 1988), on the assumption that they would merely reflect the clumping of koalas with *E. acaciiformis*.

In this study the diet of koalas was inferred from day-time observations of koalas, and feeding behaviour was not quantified independently of distribution. This could have led to errors in the estimated diet composition if koalas were using many trees in which they did not feed. Koalas do climb trees in which they do not feed, including dead trees on rare occasions (Hindell *et al.*, 1985). However, both Hindell *et al.* (1985) and Robbins and Russell (1978) found that day-time occupancy of trees furnished good estimates of the trees in which koalas had fed. Thus the foliage collected in this study from trees in which koalas were captured can be regarded as a reasonable representation of their diet.

Another possible source of error in foliage sampling is the position in the canopy from which it was taken. Aspect and shading of foliage affect the availability of sunlight for photosynthesis, and thus the relative availability of carbon to other nutrients, and the quantities of carbon-based defenses such as phenolics and essential oils (Bryant *et al.*, 1985b; Mole *et al.*, 1988). This source of error was minimized by sampling at sites in the canopy where koalas had fed. This was not always possible, but error from this source is likely to reduce the chances of detecting correlations between nutrient levels and food selection, which would make the findings from this study conservative.

### 8.4.3 Diet selection by free-living koalas

There were no measured differences between the chemical composition of the diets of lactating and non-lactating female koalas at the peak of lactational energy output. The only differences were lower phenolics and a higher ratio of nitrogen to phenolics in the diet selected by the lactating koalas after the peak of lactation. Thus, increased selectivity for higher quality diet did not play a major role in meeting the requirements of reproduction in the current study.

Although the chemical composition of the diet of koalas was not compared to available foliage at the peak of lactation, several trends were apparent during both winter and summer (Table 8.5). First, the preference of koalas for *E. acaciiformis* was clearly related to differences between *E. acaciiformis* foliage and that of the other species. Second, trends were different in summer and winter. This may be due to the availability of tips in summer; koalas may have been selecting trees on the basis of tip composition in summer, but in winter these were not as abundant (Figure 8.1).
The level of fibre and the ratio of nitrogen to fibre were the most consistent correlates of diet selection by the koalas, supporting the results of previous studies (Ullrey et al., 1981; Hume and Esson, 1993). Diet selection also correlated with the essential oil composition; koalas favouring foliage with a high proportion of volatile terpenoids and low proportion of the less volatile fraction, as suggested by previous studies of captive koalas (Betts, 1978; Hume and Esson, 1993).

Free-living koalas select individual trees within a species (Martin, 1985a; Hindell et al., 1985). In this study the pattern of selection of foliage within a tree species was complicated and seemed dependent on the negative features of the average composition of the species. The most preferred species, *E. acaciiformis*, had high levels of phenolics, and selection within this species by koalas was correlated with low levels of phenolics and/or their protein-precipitating capacity and high ratios of nitrogen to the phenolics. Like *E. acaciiformis*, the foliage of *E. radiata* had high phenolic content and activity. However, the essential oil content of *E. radiata* was very high, and in winter selection of foliage within *E. radiata* by koalas was correlated with comparatively low essential oil content. In summer, there was no apparent intra-specific selection of *E. pauciflora* or *E. radiata* on the basis of chemical composition. Again, this may be due to the availability of tips at that time; koalas may not have been selecting on the basis of the composition of mature foliage. On the other hand, *E. pauciflora* had comparatively low nitrogen content and winter selection of *E. pauciflora* foliage within the species was correlated with comparatively low nitrogen and high water content. It is possible that the correlation of low nitrogen content with tree choice within *E. pauciflora* was a spurious correlation.

It is perhaps not surprising to find such apparently weak dietary selectivity of koalas on the basis of chemical composition at the Nowendoc site. Pahl and Hume (1990) and Hume and Esson (1993) suggested that part of koala food selection is based on threshold levels of some nutrients, above which there is little selectivity on the basis of levels of those nutrients. Foliage at the Nowendoc site is the highest in levels of nitrogen, phosphorus and the ratios of these to phenolics, of foliage from any of the sites surveyed by Cork (unpubl.) in north-eastern NSW. If nutrient levels at Nowendoc are above threshold levels, selectivity on the basis of nutrient levels may be expected to be more complex than if koalas encountered foliage both above and below those threshold levels. This may partially explain why lactating females, despite their greater requirements, appeared to select similar quality foliage to that selected by non-lactataters.
8.4.4 Nutrition and lactational output

Koalas prefer tips to mature foliage (Ullrey et al., 1981; Pahl and Hume, 1990). In this study, tips were nutritionally superior to mature foliage in a number of aspects. Fewer tips were available in 1991 than in 1990 (Figure 8.1). The lactational energy output of koalas was also lower in 1991 than in 1990, both in terms of total output and intensity, and the peak energy output was 50 days later in the lactation (Chapter 5, Figure 5.11). The later peak in 1991 coincided with an increase from the previous month in nitrogen levels and decrease in fibre levels of the foliage selected by koalas. The greater availability of the preferred tips in 1990 may be the major cause of the differences in lactational output between the two years. Although data are not available on the composition of foliage in 1990, it seems likely that the level of availability of high-quality foliage determines the level of lactational output in the koala. However, the quality of foliage at the Nowendoc site is generally relatively high, so that trends in foliage selection between reproductive and non-reproductive females, and by all koalas in relation to the available foliage, were not clear cut.
CHAPTER NINE

HOME RANGES OF FREE-LIVING FEMALE KOALAS

9.1 INTRODUCTION

Home range is the area traversed by an individual animal during normal activities (Burt, 1943). Home range size is correlated with body mass and metabolic requirements over a wide range of animal taxa (McNab, 1963; Schoener, 1968; Turner et al., 1969; Harestad and Bunnell, 1979; Damuth, 1981; Harvey and Clutton-Brock, 1981; Mace and Harvey, 1983; Gompper and Gittleman, 1991). It has been suggested that home range size is determined by the interplay of energetic requirements of the animal and productivity of the area for the resource being utilized by the animal (Schoener, 1968; Mace and Harvey, 1983; Gompper and Gittleman, 1991). High energetic requirements coupled with a low productivity of the required resource results in large home ranges (e.g. large carnivores), compared with species or individuals with lower requirements (i.e. smaller size), broader resource base (e.g. omnivory), and/or greater site productivity (Gompper and Gittleman, 1991). Harvey and Clutton-Brock (1981) found that home ranges increased among species of folivorous primates as metabolic requirements increased.

Koalas have low levels of energy expenditure (Degabriele and Dawson, 1979; Nagy and Martin, 1985; this study Chapter 6) and low levels of activity (Robbins and Russell, 1978; Nagy and Martin, 1985; Mitchell, 1990b), and so could be expected to have relatively small home ranges. They also utilize an abundant resource (mature foliage), and so could be expected to have a smaller home range than a species utilizing a sparsely distributed resource such as young foliage or fruits (Milton and May, 1976; Montgomery and Sunquist, 1978), if the tree species and habitat quality are such that much of the mature foliage is acceptable. Previous estimates of the home range area of koalas vary mainly from 1.2 to 3.1 ha (Hindell and Lee, 1988; Mitchell, 1990b; Faulks, 1991), considerably smaller than the 11.4 ha predicted by McNab's (1963) relationship for mammals, although Hull (1985, cited in Mitchell, 1990b) found ranges as great as 14.4 to 15.2 ha. Mitchell (1990b) suggested that variability in the size of koala home ranges is dependent on the type of calculation used as well as on the size of patches of suitable habitat within the area; the latter explains the high values obtained by Hull (1985, cited in Mitchell, 1990b) in a highly fragmented semi-urban habitat. Koala home ranges are likely to be larger in poor
quality habitat than good quality habitat to ensure a sufficient resource base of acceptable mature foliage.

The home ranges of koalas have also been described by the number of trees used (Eberhard, 1978; Hindell and Lee, 1988, Mitchell, 1990b). However, Mitchell (1990b) suggested that this method was impractical, requiring many observations, as koalas change position only 1-3 times per 24 hours (Eberhard, 1978; Hindell et al., 1985; Mitchell, 1990b; Faulks, 1991).

Lactating female koalas have greater food requirements than non-lactating females (Chapter 6) and thus may be expected to have a greater home range area than non-lactating females, if home range area in koalas is largely determined by energy requirements. On the other hand, energy conservation (e.g. reduction in activity to partially compensate for the demands of lactation) by lactating koalas may result in smaller home ranges than those of non-lactating koalas. This chapter investigates variation in home range of female koalas during lactation, based on radio-location of females over the period of greatest energetic demand.

9.2 METHODS

9.2.1 Home range estimation

Locations of individual koalas were determined by radio-location with visual confirmation and triangulation to marked grid points as described in Chapter 3. The locations were highly accurate; repeated determinations of the position of individual trees were within 5 m of each other. The koala's location was measured as being the position of the base of the tree it occupied.

Locations were determined in conjunction with the measurements described in the earlier chapters. To minimize the degree of autocorrelation among the locational data for any one koala, a maximum of one location daily was included in the analyses. It was noted that koalas often crossed their range within a day, so daily fixes should not have been highly autocorrelated (Swihart and Slade, 1985).

Home ranges were determined by the minimum convex polygon (MCP) and harmonic-mean (Dixon and Chapman, 1980) methods, calculated using the MCPAAL computer program (Stuwe and Blohowiak, 1985). Although the minimum convex polygon method included large areas of open paddock over which koalas travelled between patches of trees, it was used because it is the only method that is truly comparable between studies, and least affected by sample size and autocorrelation.
(Harris et al., 1990). Results for four isopleths of the harmonic home range are presented; 95% and 50% for comparison with most other studies of home range, 90% for comparison with previous studies of koalas, and 70% to delineate areas of core usage (as determined below).

The size of the estimated home range is positively correlated with the number of observations; increasing the number of observations increases the chances of including outlying locations (Harris et al., 1990). Above a certain number of observations the home range area will often approach an asymptote (Jaremovic and Croft, 1987; Harris et al., 1990). To determine the appropriate number of observations required to define the home range of koalas in this study, four koalas were selected from among those with more than 40 observations over the entire study. Home ranges were calculated for each of the four animals using the full set of observations, as well as for reduced data sets with observations deleted randomly to the closest multiple of five, then by fives to a minimum of five. The resulting relationship between home range size and number of observations was plotted and the tendency toward an asymptote assessed visually (Harris et al., 1990).

The full data sets for the same four animals were used to estimate the appropriate isopleth to define core areas within the harmonic-mean home ranges. The area inside the isopleth was plotted against the percentage of observations represented by the isopleth (from 10 to 95%) and the isopleth corresponding to the most rapid change of slope of the relationship was taken to define an animal's core area (Harris et al., 1990).

9.2.2 Comparison of home ranges of lactating and non-lactating koalas

Home range areas of adult female koalas were compared using observations recorded between May and December of any one year. This time period was selected because the demands of lactation were greater during those months than in the rest of the year (Chapter 5). Because the home range area estimated depended on the number of observations (see Results), the number of locations of each koala was adjusted to the lowest number in the dataset (15 observations) by randomly deleting locations. To compare home ranges of lactating and non-lactating koalas, estimates from both years were used in the same analysis. In the cases where estimates of home range were available from both years for the one animal, the estimate of non-lactating range was used; in the two cases where the koala lactated in both years, one was selected at random.
9.2.3 Movement

Daily movements of adult female koalas were monitored from observations every 4-8 h during measurement of digesta passage rates in August/September 1991, close to the period of peak lactation. The distance travelled was calculated from the coordinates of trees they used, assuming that they travelled in a straight line between trees.

9.2.4 Statistical

Differences between means were tested with Student's t-test or the non-parametric Mann-Whitney U-test in the cases whenever Bartlett's test showed that the variances were heterogeneous between groups (Wilkinson, 1989).

9.3 RESULTS

9.3.1 Estimation of core areas

The harmonic-mean isopleth distinguishing core areas of the home range varied among the four animals considered. The slope of the area/isopleth curve changed most rapidly at the 70 % level for K2, 80 % for K27, and around 60 % for K36 and K65 (Figure 9.1). The 70 % harmonic isopleth was chosen to represent core areas of the home range as it was closest to the mean of the four animals.

9.3.2 The relationship between calculated home range and the number of observations

The home range area calculated depended on the number of observations on which it was based. The area inside the minimum convex polygon was the only measure to show any tendency toward an asymptote within the range of observations available (Figure 9.2a). All the harmonic-mean measures of home range area (Figure 9.2) increased almost linearly with increasing number of observations.

9.3.3 Reproductive status and home range

There was a high degree of variability among individuals in all estimates of home range areas for female koalas, though the greatest variability was in the area of the minimum convex polygons (Table 9.1). This was mainly due to the inclusion in the polygon of large areas of open paddock between patches of trees used by koalas. These open areas were not included in the harmonic-mean home ranges; rather the
patches were shown as separate centres of activity. Each home range had several such discrete centres of activity, even at the 95 % harmonic-mean isopleth level.

The minimum convex polygon and core area (70 % harmonic-mean isopleth) estimates of the home ranges of non-lactating females were significantly larger than those of lactating females. The greater area in the 95 % and 50 % harmonic-mean isopleths of the non-lactating females approached significance (Table 9.1).

Both lactating and non-lactating home ranges were obtained for three koalas. In each case all the non-lactating estimates of home range were greater than the lactating values, although only the area inside the 50 % harmonic-mean isopleth was significantly greater (Paired t-test, Mean diff. = 0.14 ± 0.03 ha, P=0.020), largely due to the high variances. The sample size of three was too small to yield useful results with the non-parametric Wilcoxon rank sum test.
Table 9.1 Estimation of core harmonic-mean (HM) home range isopleths. a) K2, b) K27, c) K36, d) K65.
Figure 9.2. The dependence of home range area of K36 on the number of observations (a) minimum convex polygon, (b) 90% harmonic-mean isopleth, (c) 70% harmonic-mean isopleth, (d) 50% harmonic-mean isopleth.
Table 9.1 Home range areas (ha) of adult female koalas (based on 15 observations).

<table>
<thead>
<tr>
<th>Year</th>
<th>Status</th>
<th>Koala</th>
<th>MCP¹</th>
<th>Harmonic-mean isopleths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>95 %</td>
<td>90 %</td>
</tr>
<tr>
<td>1990</td>
<td>L</td>
<td>2</td>
<td>2.12</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>3.25</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>1.75</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27</td>
<td>1.89</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36</td>
<td>14.81</td>
<td>4.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51</td>
<td>3.00</td>
<td>3.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57</td>
<td>1.07</td>
<td>0.30</td>
</tr>
</tbody>
</table>

|      |        |       | 3.98 | 1.72 | 1.21 | 0.45 | 0.17 |
|      |        |       | (4.83) | (1.69) | (1.28) | (0.62) | (0.20) |
| NL   | 11     | 12.66 | 3.21 | 1.99 | 0.95 | 0.35 |
|      | 33     | 5.02  | 2.85 | 1.32 | 0.34 | 0.19 |

|      | Mean   |       | 8.84 | 3.03 | 1.66 | 0.65 | 0.27 |
|      |        |       | (5.40) | (0.25) | (0.47) | (0.43) | (0.11) |
| 1991 | L      | 11    | 12.17 | 1.63 | 1.46 | 0.86 | 0.23 |
|      |        | 27    | 2.73  | 1.02 | 0.81 | 0.28 | 0.09 |
|      |        | 34    | 4.00  | 0.85 | 0.78 | 0.27 | 0.17 |
|      |        | 36    | 8.27  | 3.99 | 3.26 | 1.15 | 0.42 |
|      |        | 65    | 4.41  | 1.06 | 0.74 | 0.23 | 0.10 |
|      |        | 82    | 1.92  | 0.85 | 0.78 | 0.27 | 0.17 |

|      | Mean   |       | 5.58 | 1.57 | 1.31 | 0.51 | 0.20 |
|      |        |       | (3.90) | (1.22) | (1.00) | (0.39) | (0.12) |
| NL   | 2      | 3.20  | 2.13 | 1.68 | 0.59 | 0.18 |
|      | 14     | 7.60  | 3.02 | 2.04 | 1.10 | 0.43 |
|      | 19     | 5.91  | 1.41 | 1.28 | 0.67 | 0.23 |
|      | 20     | 17.16 | 2.89 | 2.18 | 1.34 | 0.80 |
|      | 62     | 15.30 | 7.07 | 5.99 | 1.86 | 0.71 |

|      | Mean   |       | 11.49 | 3.60 | 2.87 | 1.24 | 0.54 |
|      |        |       | (5.57) | (2.43) | (2.12) | (0.50) | (0.26) |
| Overall | Mean |       | 3.58  | 1.55 | 1.24 | 0.39 | 0.19 |
|        | (N=8) |       | (2.17) | (1.41) | (1.16) | (0.38) | (0.19) |
| NL    | 9.55  | 3.23  | (N=7) | (5.45) | (1.81) | (1.64) | (0.51) | (0.25) |
|        |       |       |       |       |       |       | 0.015³ | 0.065 | 0.147 | 0.024 | 0.075 |

¹ MCP = minimum convex polygon.
² Probability from Student's t-test.
³ Mann-Whitney U-test result as variances were heterogeneous.

L = lactating and NL = non-lactating females.

<table>
<thead>
<tr>
<th>Year</th>
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<th>MCP¹</th>
<th>Harmonic-mean isopleths</th>
</tr>
</thead>
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<td>90 %</td>
</tr>
<tr>
<td>1990</td>
<td>L</td>
<td>2</td>
<td>2.12</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>3.25</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>1.75</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27</td>
<td>1.89</td>
<td>1.42</td>
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<tr>
<td></td>
<td></td>
<td>36</td>
<td>14.81</td>
<td>4.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51</td>
<td>3.00</td>
<td>3.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57</td>
<td>1.07</td>
<td>0.30</td>
</tr>
</tbody>
</table>

|      | Mean   |       | 3.98 | 1.72 | 1.21 | 0.45 | 0.17 |
|      |        |       | (4.83) | (1.69) | (1.28) | (0.62) | (0.20) |
| NL   | 11     | 12.66 | 3.21 | 1.99 | 0.95 | 0.35 |
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|      | Mean   |       | 8.84 | 3.03 | 1.66 | 0.65 | 0.27 |
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| 1991 | L      | 11    | 12.17 | 1.63 | 1.46 | 0.86 | 0.23 |
|      |        | 27    | 2.73  | 1.02 | 0.81 | 0.28 | 0.09 |
|      |        | 34    | 4.00  | 0.85 | 0.78 | 0.27 | 0.17 |
|      |        | 36    | 8.27  | 3.99 | 3.26 | 1.15 | 0.42 |
|      |        | 65    | 4.41  | 1.06 | 0.74 | 0.23 | 0.10 |
|      |        | 82    | 1.92  | 0.85 | 0.78 | 0.27 | 0.17 |

|      | Mean   |       | 5.58 | 1.57 | 1.31 | 0.51 | 0.20 |
|      |        |       | (3.90) | (1.22) | (1.00) | (0.39) | (0.12) |
| NL   | 2      | 3.20  | 2.13 | 1.68 | 0.59 | 0.18 |
|      | 14     | 7.60  | 3.02 | 2.04 | 1.10 | 0.43 |
|      | 19     | 5.91  | 1.41 | 1.28 | 0.67 | 0.23 |
|      | 20     | 17.16 | 2.89 | 2.18 | 1.34 | 0.80 |
|      | 62     | 15.30 | 7.07 | 5.99 | 1.86 | 0.71 |

|      | Mean   |       | 11.49 | 3.60 | 2.87 | 1.24 | 0.54 |
|      |        |       | (5.57) | (2.43) | (2.12) | (0.50) | (0.26) |
| Overall | Mean |       | 3.58  | 1.55 | 1.24 | 0.39 | 0.19 |
|        | (N=8) |       | (2.17) | (1.41) | (1.16) | (0.38) | (0.19) |
| NL    | 9.55  | 3.23  | (N=7) | (5.45) | (1.81) | (1.64) | (0.51) | (0.25) |
|        |       |       |       |       |       |       | 0.015³ | 0.065 | 0.147 | 0.024 | 0.075 |

L = lactating and NL = non-lactating females.

¹ MCP = minimum convex polygon.
² Probability from Student's t-test.
³ Mann-Whitney U-test result as variances were heterogeneous.
9.3.4 Daily movements of female koalas near the period of peak lactation

There were no significant differences between the daily movements of lactating and non-lactating females (Table 9.2). The female koalas used an average of $1.9 \pm 0.6$ trees per day, and travelled an average of $163 \pm 76$ m horizontally per day.

### Table 9.2 Daily movement of adult female koalas near the period of peak lactation.

<table>
<thead>
<tr>
<th>Koala</th>
<th>Trees (d$^{-1}$)</th>
<th>Distance (m.d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1.4</td>
<td>150</td>
</tr>
<tr>
<td>27</td>
<td>2.7</td>
<td>221</td>
</tr>
<tr>
<td>36</td>
<td>1.9</td>
<td>275</td>
</tr>
<tr>
<td>65</td>
<td>1.2</td>
<td>81</td>
</tr>
<tr>
<td>Non-lactating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>201</td>
</tr>
<tr>
<td>14</td>
<td>2.5</td>
<td>62</td>
</tr>
<tr>
<td>19</td>
<td>2.2</td>
<td>151</td>
</tr>
<tr>
<td>20</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactating</td>
<td>1.8 ± 0.7</td>
<td>182 ± 84</td>
</tr>
<tr>
<td>Non-lactating</td>
<td>1.9 ± 0.5</td>
<td>138 ± 70</td>
</tr>
<tr>
<td>P</td>
<td>0.762</td>
<td>0.500</td>
</tr>
<tr>
<td>Mean</td>
<td>All 1.9 ± 0.6</td>
<td>163 ± 76</td>
</tr>
</tbody>
</table>

9.4 DISCUSSION

9.4.1 Methods

Home ranges of koalas in this study were not fully defined by the number of observations made, as there was no obvious tendency toward an asymptote of home range area (Figure 9.2). Therefore, calculation of the full home range of koalas at the Nowendoc study site requires more than 75 independent observations (the greatest number obtained in this study). Similarly, Mitchell (1990b) found that areas of the harmonic-mean home ranges were positively correlated with the number of observations at the 95 % isopleth level (but not at the 90 % isopleth). The average number of observations in that analysis was 39. Mitchell (1990b) also found large asymmetries between the ranges of individuals in different seasons. Similar seasonal range shifts by koalas at the Nowendoc site could account for the observed sample-size dependence. Additional observations would have a high chance of being drawn
from a different seasonal range, thus increasing the number of outliers and so the number of observations required to reach an asymptote.

The cumulative number of trees used by koalas on French Island approached asymptotes up to 300 trees (Mitchell, 1990b), so using trees to define home range would require considerably more observations than were made in this study. Mitchell (1990b) also suggested that at his study area, koalas may over time have used all the trees available to them within their home range.

Because of the dependence of the home ranges on sample size it was necessary to calculate all home ranges for comparison within the study using a standard number of observations. Both Mitchell (1990b) and Hindell and Lee (1988) used varying numbers of observations to calculate koala home ranges (Mitchell 11-94 obs., Hindell and Lee >15 obs.). This means that the home ranges from this study are an index of home range, and possibly considerably smaller than the true ranges of the koalas. They are not strictly comparable to the previous studies, but comparisons made within the study should be valid.

Minimum convex polygons were included largely to allow comparison with other studies (Harris et al., 1990), as well as giving an indication of the total area that may be traversed. Because they included large areas of open paddock not utilized except during movements, the minimum convex polygons do not indicate the area of suitable habitat required by koalas, but because the energetic expenditure of locomotion is proportional to the distance moved (Taylor et al., 1982), they do give an indication of the energy expenditure that might be required to move around in the range. The harmonic-mean method provided a better estimate of the actual area regularly used by koalas; it did not include large areas of open paddocks, but often consisted of several patches and thus did not indicate the likely energy expenditure involved in moving regularly between patches. Of the commonly used methods, Boulanger and White (1990) considered the 95 % harmonic-mean isopleth to provide the best estimate of home range. Harmonic-mean ranges also provided an estimate of the area of core usage by koalas, indicating the most important sections of the home range.

Areas of home ranges measured in this study, 1.24 ± 1.16 ha for lactating females and 2.35 ± 1.64 ha for non-lactating females (as defined by the 90 % harmonic-mean isopleth), were similar to those in two previous studies. Hindell and Lee (1988) estimated the average home range of female koalas to be 2.08 ± 0.86 ha, and Mitchell (1990b) calculated it to be 1.18 ± 0.65 ha. It is surprising that the home ranges measured in the fragmented habitat at Nowendoc were not greater than those reported by Hindell and Lee (1988) and Mitchell (1990b) in less fragmented habitat with a
greater population density (3.9-8.9 koalas per hectare compared with about 0.5 at Nowendoc). However, because the home range areas were positively correlated with sample size and the average numbers of observations in their studies were greater, it is possible that the true home ranges of koalas at Nowendoc were actually larger than on French Island and in the Brisbane Ranges. True home ranges of female koalas at Nowendoc for the full year may be from around 4 to 12 ha.

9.4.2 Lactation and home range

Lactating female koalas at the Nowendoc site had smaller home ranges than non-lactating females. The largest difference was in the minimum convex polygon ranges, where non-lactating females covered almost three times as much area as lactators. This was largely due to the inclusion of greater areas of open paddock in the ranges of non-lactating females, indicating that the lactators included fewer separate patches of suitable habitat in their home range than did non-lactators, thus decreasing the energetic expenditure of travel within their range and possibly also decreasing their susceptibility to terrestrial predators. The core areas (70% harmonic isopleth) used by lactators were also smaller than those of non-lactators, indicating a greater concentration of activity within core areas. In the paired comparison of home ranges of individuals during lactation and during years in which they didn't lactate, the home ranges during lactation were always smaller than the non-lactating ranges. This also strongly suggested a contraction of range during lactation.

It is tempting to postulate that the lactating females concentrate their activities around patches of the most suitable habitat, maximising the quality of their food and minimizing the energy and risks associated with changing trees. However, at present there is no evidence to support this hypothesis. Lactating koalas did not use less trees or travel smaller distances per 24 hour period in this study than did non-lactating females, nor was the quality of their food measurably greater (Chapter 8). Nevertheless, the contraction of home ranges by lactating koalas suggests that further study of behaviour, movements, and the makeup (floristically, physically and nutritionally) of core areas during lactation may shed further light on the habitat requirements of lactating female koalas.

9.4.3 Could lactating female koalas compensate for the energy requirements of lactation by reducing activity?

Female koalas partially compensated for the energy requirements of reproduction by reducing other components of their energy budget (Chapter 6). The total energy compensation observed in 1990 was 412 kJ.d⁻¹ (Table 6.10).
However, the total activity budget of an average 6 kg female koala is around only 82 kJ.d\(^{-1}\) (Table 9.3), only 5 % of the field metabolic rate and 20 % of the observed compensation. Lactating females consume more foliage than non-lactating females, so are unlikely to be able to reduce the portion of their activity budget devoted to feeding. Even if lactating koalas reduced their locomotory requirements to zero, the maximum energetic saving would be 23 kJ.d\(^{-1}\), only 6 % of the energy required to produce milk. Therefore, reduction in the energy requirements of activity in lactating females could not compensate for a large proportion of the energy requirements for lactation. Alternatively, contraction of home range during lactation may serve more to reduce exposure to terrestrial predators such as man, dingoes and foxes, rather than to compensate for the energy requirements of lactation.

Table 9.3 Daily activity budget\(^{1,2}\) of a 6kg koala.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Time (h)</th>
<th>Expenditure (kJ)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting</td>
<td>19</td>
<td>RMR</td>
<td>Nagy and Martin (1985)</td>
</tr>
<tr>
<td>Active</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>22</td>
<td>Nagy and Martin (1985)</td>
</tr>
<tr>
<td>Feeding</td>
<td>3</td>
<td>36</td>
<td>Nagy and Martin (1985)</td>
</tr>
<tr>
<td>Locomotion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizontal (300 m)</td>
<td>6</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>Vertical (45 m)</td>
<td>17</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>82</strong></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Values of activity are approximate and were adjusted to represent relatively high expenditure.

\(^2\) Energetic equivalents of activity taken from Robbins (1983).

RMR is resting metabolic rate.
CHAPTER TEN

GENERAL DISCUSSION AND SYNTHESIS: HOW DOES AN ARBOREAL FOLIVORE MEET THE ENERGY REQUIREMENTS FOR REPRODUCTION?

Reproduction places large energetic demands on female mammals. The most demanding part of reproduction is usually the peak of lactation, and demands are met primarily by increases in dietary energy intake (Chapter 1). Therefore, the rate of allocation of energy to reproduction may be limited primarily by limits to dietary energy intake (Kenagy et al., 1990). Arboreal folivores in general, and the koala in particular, face allometric constraints to their intake of dietary energy (Chapter 2). In consequence, the koala exists on a fine energy balance (Cork and Sanson, 1990), and manages largely due to its energetically conservative characteristics (Chapter 6; Degabriele and Dawson, 1979; Nagy and Martin, 1985).

Maximum output may be limited by allometric constraints on the female koala's ability to extract nutrients from *Eucalyptus* foliage, but the requirements of the young and the necessity of producing a self-sufficient offspring within the time before the next reproductive season set a lower limit to the expenditure required for successful reproduction. This creates a narrow window of viable energy expenditures bounded above by limits to intake and below by requirements and minimum size for folivory of the young. This study has shown that koalas meet the energy requirements for reproduction primarily by extending the period of lactation so that reproductive demands even at the peak of lactation fit below the upper bounds to energy intake for the koala, while raising intake above the lower limit set by the requirements of the young.

The composition of koala milk was within the ranges found in other marsupials (Chapter 4, Table 4.2). However, the patterns of changes in composition differed in some respects (Green, 1984; Green and Merchant, 1988). Lipid was high throughout lactation and was the major source of energy even early in lactation. Consequently, there was no obvious switch from carbohydrate to lipid as an energy source for juvenile koalas, as seen around the time of pouch exit in macropods (Janssens and Ternouth, 1987). Lipid levels in milk were higher than in the other marsupial folivores (Cowan, 1989; Munks et al., 1991) and closer to those of the wombat (Green, 1984), the closest extant relative of the koala; this may indicate an effect of phylogeny on milk composition. Changes in carbohydrate structure throughout lactation in the koala were similar to those in the tammar wallaby (Messer and Green,
1979; Green and Merchant, 1988), although the disaccharide lactose rather than monosaccharides was the major carbohydrate late in lactation. This was a pattern seen also in other folivorous marsupials such as ringtail and brushtail possums (Crisp et al., 1989a; Munks et al., 1991).

Peak milk energy production in the koala was the lowest measured for any mammal (Table 5.4), but because of the long duration of lactation, the total requirement of the mother for metabolizable energy to support reproduction was within the range reported for other mammals. Thus, koalas meet the daily requirements of reproduction by spreading the load of reproduction over a long period, reducing daily requirements (Figure 10.1).

Figure 10.1 Metabolizable energy requirements for reproduction in five mammals. $A_S$, $A_C$, $A_T$, $A_R$, and $A_K$ denote the total requirements for reproduction (areas under the curves) in sheep, cattle, the tammar wallaby, the common ringtail possum and the koala respectively (adapted from Cork and Dove, 1989; ringtail possum from Munks, 1990; koala from this study using data from 1991).

There were considerable differences in the output of milk energy by female koalas between the two years of the study (Chapter 5). Total milk production was greater in 1990 than 1991 (Chapter 5). Consequently, both total and peak allocations of energy to reproduction by female koalas in 1991 were only 65% of those in 1990. The
difference in allocation of energy to reproduction between the two years may have been due to the greater availability of preferred young foliage in 1990 than 1991 (Chapter 8), which presumably resulted in a higher quality diet in the first year of the study. However, despite the differences in milk energy production between the two years, there were no differences in growth rates of the young. This suggests that growth of the young in 1990 may have been limited by some other factor, such as the intake of protein rather than energy, as protein intake was similar between years.

The narrow margin between energy intake (mainly as milk) and the energy expenditure (FMR) of juvenile koalas around the time of permanent pouch exit in 1991 (only 7-30 kJ.d\(^{-1}\) metabolizable energy), suggests that this is potentially a period of nutritional stress for the young. As little or no foliage was consumed by the young at that age, the milk energy output in 1991 must have been close to the minimum required to raise a young to weaning without extending lactation into the next breeding season and consequently foregoing the opportunity to breed again. Thus, requirements of the young set the lower boundary of the window of possible maternal energy output for successful reproduction, while allometric constraints to extraction of energy from foliage probably set the upper boundary.

Koalas met at least 90 % of the demands of their prolonged lactation by increasing food energy intake (Chapter 6). They utilized very little, or no, body energy stores to meet lactational requirements (Chapter 6). Food intake of lactating females at the time of peak lactation was 27 % greater than that of non-lactating females during the same period. This increase in food intake at peak lactation was not associated with a decrease in retention times of digesta within the gut (Chapter 7) or measurably increased dietary selectivity (Chapter 8). The capacity of the gut for fluid digesta was 33 % greater (as a proportion of body mass) in lactating females than non-lactating females, allowing greater intake of foliage without decreasing the mean retention time of fluid and fine particulate digesta and thus avoiding the depression in digestibility and elevated loss of nitrogen in faeces associated with rapid transit.

Resting metabolic rates of lactating females were greater than those of non-lactating females, but the increase was only 42 % of that expected due to energy expenditure during synthesis of milk (Chapter 6). Field metabolic rates of both lactating and non-lactating females varied seasonally, with a nearly twofold increase from summer to winter (Chapter 6). However, the field metabolic rate of lactating females was no greater than that of non-lactating females at the time of peak lactation, despite the increased energy expenditure associated with synthesis and export of milk and with acquisition and processing of food (specific dynamic action). These extra energy
expenditures of lactating females could be expected to increase their energy budget by 270-420 kJ.d\(^{-1}\) (in 1991 and 1990 respectively) which may be expected to have raised their field metabolic rate above the 95\% confidence limits of that of the non-lactating females. That this did not occur suggests that lactating females compensated for part of the energy requirements of reproduction by reducing other aspects of their total energy expenditure.

Although lactating females had smaller home ranges than did non-lactating females (Chapter 9), their low normal level of activity means that the reduction in home range size could not have compensated for any substantial proportion of reproductive energy expenditure. It is more likely that the reduction in home range served to reduce exposure of the potentially vulnerable females with back-young to terrestrial predators.

Some of the compensation for reproductive energy demands may have occurred in thermoregulatory expenditure. By carrying a juvenile on the back or belly, lactating females (at the time of peak lactation) were estimated to reduce their surface area by about 10\%, saving up to 108 kJ.d\(^{-1}\) in thermoregulatory expenditure. However, some of the compensation was due to a reduction of some component of the resting metabolic rate, as it rose by only 42\% of the expected increase. Thus there were multiple sources of compensation for reproductive energy expenditure.

In conclusion, koalas met the energy demands for reproduction in two ways. First, by spreading lactation over a long period they minimized daily energetic demands. Compensation for reproductive energy expenditure by reduction of other components of resting and field metabolic rates reduced daily reproductive demands even further. Second, by increasing gut capacity they were able to increase food energy intake to meet energy demands.
References

Agricultural Research Council (1980). The nutrient requirements of ruminant livestock. Farnham Royal: Commonwealth Agricultural Bureaux.


Cork, S.J. (in prep). Digestive constraints on dietary scope in small and moderately-small mammals: How much do we really understand?


Foley, W.J. and Cork, S.J. (1992) Utilization of fibrous diets in small herbivores- how far can the rules be "bent". TREE 7: 159-162


Maclean, S., Foley, W.J., Davies, N.W., Brandon, S., Duo, L. and Blackman, A.J. (in prep.). Metabolic fate of dietary terpenes from *Eucalyptus radiata* in the common ringtail (*Pseudocheirus peregrinus*).


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Appendix 1. The composition of foliage, from trees in which koalas were found, throughout 1991 (means ± SE).

a) Dry matter and neutral detergent fibre (NDF). Dry matter as a percentage of fresh weight and NDF as a percentage of dry weight. b) Nitrogen as a percentage of dry weight.
Appendix 1 (cont.) c) Protein-precipitating capacity and total phenolic content of foliage, as mg.g\(^{-1}\) of dry matter. d) The ratios of nitrogen to: fibre (N:NDF), protein-precipitating capacity (N:PP), and total phenolics (N:TP) in g.g\(^{-1}\).
Appendix 1 (cont.) e) Essential oil content of foliage (% v/w). f) Composition of the essential oil, abbreviated as in Table 8.5.