Milk composition of the Tasmanian pademelon (*Thylogale billardierii* Desmarest) (Macropodoidea: Marsupialia) in captivity

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Abstract. This is the first study of milk composition in any member of the wallaby genus *Thylogale*. Milk samples ($n = 44$) were collected after oxytocin injection from 12 females carrying pouch young aged 2–30 weeks followed by sampling of the mothers until weaning by Week 39. The lactation period could be partitioned into early (Weeks 2–20), mid (Weeks 21–30) and late (>30 weeks) stages. Although milk concentrations changed little during lactation the energy content increased almost 4-fold from $540 \pm 39$ kJ (100 mL)$^{-1}$ (mean ± s.e.) to $1908 \pm 102$ kJ (100 mL)$^{-1}$. Carbohydrate concentrations decreased from $13.3 \pm 0.1$ g (100 mL)$^{-1}$ (early lactation) to $10.9 \pm 0.9$ g (100 mL)$^{-1}$ in mid-lactation, falling to $4.8 \pm 0.9$ g (100 mL)$^{-1}$ in late lactation. Lipid increased from $6.3 \pm 1.1$ g (100 mL)$^{-1}$ to $12.5 \pm 4.1$ g (100 mL)$^{-1}$, reaching $31.4 \pm 5.0$ g (100 mL)$^{-1}$ in late lactation. Protein increased from $3.3 \pm 0.1$ g (100 mL)$^{-1}$ to $9.7 \pm 1.6$ g (100 mL)$^{-1}$ in mid-lactation to $14.0 \pm 1.5$ g (100 mL)$^{-1}$ in late lactation. This relatively high level of lipid and protein in late lactation may be the cause of the more rapid growth and, hence, shorter pouch life of the Tasmanian pademelon (*Thylogale billardierii*) compared with the well studied tammar wallaby (*Macropus eugenii*).

Introduction

The Tasmanian pademelon (*Thylogale billardierii*) is a small to medium-sized wallaby (3.5–6.0 kg) found only in Tasmania. In the wild the pademelon has a restricted breeding season, with most births occurring in autumn; after ~7 months the young leaves the pouch in early summer (Rose and McCartney 1982\textsuperscript{a}, 1982\textsuperscript{b}). In other aspects of the reproductive cycle, the pademelon conforms to the usual pattern found in other kangaroos, wallabies and rat-kangaroos: the duration of the oestrous cycle is 30.3 days; after a relatively short gestation period (30.2 days) the young climbs unaided into the pouch, attaching to one of the four teats that has not been used recently by a sibling; shortly after giving birth the mother comes into oestrus, mates and may produce a fertilised egg that will develop to the blastocyst stage but for most of the period that the pouch is occupied the blastocyst will remain in the uterus in embryonic diapause; should the young in the pouch be lost or removed experimentally the inactive blastocyst will develop and be born 27–28 days later; however, if the young in the pouch develops naturally, it will be replaced on the same night by a neonate resulting from the previous activation of the diapausing young (again, the mother will mate at *post partum* oestrus).

It seems likely that in the pademelon secretions of prolactin resulting from lactation for the pouch young inhibit the development of the diapausing embryo in a similar way to that in the tammar wallaby (*Macropus eugenii*) and Bennett’s wallaby (*Macropus rufogriseus*) (Tyndale-Biscoe and Renfree 1987). Rose (1997) has demonstrated that Tasmanian pademelon embryos in diapause will commence development almost immediately after a single injection of a dopamine agonist, bromocriptine (CB154), which is known to reduce prolactin concentrations in plasma. The ‘premature’ birth of these young sometimes coincides with early emergence from the pouch (‘pouch vacation’: Rose 1986) of their older sibling (Rose 1997).

Rose et al. (1999) took the study of the reproduction of *T. billardierii* further and noted that this species’ reproduction was very similar in most parameters to that of the very well studied tammar wallaby. It differed in two major parameters: (1) it was capable of breeding throughout the year, and (2) the duration of pouch life of 202 days was much shorter than that of the tammar (~240) although both species left the pouch at a similar mass. As such, it was suggested that it was a potentially useful laboratory species. This paper provides additional information on the lactation of this species.

Lactation is relatively far more important to marsupial young than to those of eutherian mammals in that it lasts proportionally much longer than gestation. Analysis of the milk of several kangaroos has been reported and all have shown that the concentration of the milk increases over the duration of pouch occupancy and that there are both quantitative and qualitative changes in milk composition within and between species. This paper reports for the first time on changes in milk composition in a species of *Thylogale*. 

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Methods and Materials

Originally, pademelons were caught in the wild (Rose and McCartney 1982a) and formed a breeding colony. Their husbandry was as in Rose and McCartney (1982a); however, during the cooler months when insufficient grass was available the diet was supplemented with solid dog food (Uncle Bens®). Previously, we have shown that, in captivity, pademelons will breed throughout the year and produce new young after loss or removal of the pouch young (Rose and McCartney 1982a, 1982b).

Milk collection

Forty-four milk samples were obtained from 12 captive female pademelons during Weeks 2–39 of lactation (Table 1). Most pademelons were sampled on 3–4 occasions. Mothers were held in a hessian bag and the young removed and placed in a humidicrib kept at 35°C. After 4–6 h, an injection of oxytocin (Syntocinon: 1 IU kg⁻¹) was administered and the mammary teat cleaned and the milk (0.5–1.0 mL) collected into vials. Gentle massage of the teat was required on occasions in order to establish milk flow. Milk was then frozen and maintained at −20°C until analysis. The pouch young were weighed at the time of milking and their head length measured with vernier callipers. The head length was used to estimate the age of the young as in Rose and McCartney (1982b). Once young had left the pouch they were not caught or measured again.

Milk analysis

Dry weight, lipid, protein and carbohydrates were estimated with the techniques used previously in our laboratory (Crowley et al. 1988; Smolenski and Rose 1988; Rose et al. 2003). The total solids content of the milk was estimated by freeze-drying weighed quantities (50–75 µL) of whole milk. Total protein of whole milk was estimated by the dye-binding method of Bradford (1976) with Coomassie Blue dye and bovine serum albumin as the standard. Total carbohydrate was measured as hexose using the phenol–sulfuric method (Dubois et al. 1956), as modified by Messer and Green (1979).

Total lipid was estimated by the creamatocrit procedure (Lucas et al. 1978). On centrifugation, the top layer of the milk sample was clear and oily, and the adjacent layer was white and opaque. However, qualitative thin-layer chromatography on silica gel (petroleum ether (60–80°C fraction)/acetone/glacial acetic acid, 89:11:31) indicated that both layers had the same constituents, predominantly triglyceride with a small amount of phospholipid. Therefore, the total thickness of the lipid layer was used in calculating the creamatocrit measure. Calibration of the creamatocrit against the Roese–Gottlieb method (Horwitz 1980), using five samples of pademelon milk, yielded a linear calibration equation:

\[ y = 0.48 + 1.47x, \]

where \( x \) is fat concentration (g (100 mL)⁻¹) and \( y \) is creamatocrit (%) \( (r = 0.96) \).

Results

There were changes in both milk composition and milk concentration. During the early period of lactation from Week 2 until Week 20 there were few changes in milk composition but subsequently there were major changes in all parameters.

Total solids increased slowly from 18.9 ± 0.5% (w/w) until an increase in Weeks 21–30 to 25.5 ± 1.6% (w/w), increasing to 31.9 ± 1.2% (w/w) in late lactation.

Table 1. The lactational periods of twelve pademelons from which milk samples were obtained

<table>
<thead>
<tr>
<th>Stage of lactation</th>
<th>No. samples (( n ))</th>
<th>Range of ages of young (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>21</td>
<td>2–20</td>
</tr>
<tr>
<td>Mid</td>
<td>15</td>
<td>21–30</td>
</tr>
<tr>
<td>Late</td>
<td>8</td>
<td>31–39</td>
</tr>
</tbody>
</table>

Fig. 1. The change in concentration (g (100 mL)⁻¹ of milk) of the three major constituents of Tasmanian pademelon milk (carbohydrate, lipid and protein) over the three periods of lactation.

Fig. 2. The percentage contribution of the major constituents of Tasmanian pademelon milk to the total solids fraction.
Carbohydrate gradually changed from 13.3 ± 1.0% (w/w) in early lactation to 10.9 ± 0.9% (w/w) at mid-lactation before dropping substantially to 4.8 ± 0.9% (w/w) in late lactation. Protein levels were stable at 3.3 ± 0.1% (w/w) for most of early lactation, rising to 9.7 ± 1.6% in mid-lactation, and rising further to 14.0 ± 1.5% (w/w) in late lactation. Lipid remained low (6.3 ± 1.1%) during early lactation followed by an increase to 12.5 ± 4.1% (w/w) in mid-lactation and further increasing to 31.4 ± 5.0% (w/w) in late lactation. These data are presented in Fig. 1. The relative percentage contribution of the three milk constituents in the solid fraction are represented in Fig. 2; carbohydrate contribution drops and lipid contribution increases throughout the three lactation stages.

Milk energy content was calculated from the averaged measured milk components using energetic equivalents of 37.0 kJ g⁻¹, 17.0 kJ g⁻¹, and 16.0 kJ g⁻¹ for lipid, protein and carbohydrate, respectively (Thomas and Corden 1977). The levels increased from 5.4 ± 0.4 kJ mL⁻¹ to 11.9 ± 0.14 kJ mL⁻¹ to 19.1 ± 1.03 kJ mL⁻¹ (Fig. 3). There was a rapid increase in body mass from Week 20 (Fig. 4), which is the start of mid-lactation, and the milk energy changes appear to parallel the mass increase of the young ($R^2 = 0.92$) (Fig. 5).

**Discussion**

Due to the timing of this study, fewer early and weaning samples were obtained. However, the milk of *T. billardieri* exhibits qualitative and quantitative changes similar to those observed in other marsupials. The lactation period lasts 202 days, similar to those of *Petrogale penicillata* and *Macropus parma* but is considerably shorter than the 250 days of lactation of *M. eugenii*, although they are all
similar-sized wallabies (Rose 1989). The lactation period could be divided into stages according to the qualitative changes that occurred: early, mid and late stages of lactation could be discerned.

Quantitative changes in three major components of milk (lipids, carbohydrates and proteins) were recorded through the three stages of lactation. These results compare with other marsupials (particularly the tammar wallaby) in which lipid increases corresponded with carbohydrate decreases (Green et al. 1980). High levels of lipid and protein at this stage in the pouch life of *Thylogale* young provided a milk high in energy as well as 'building' material, which may explain why pademelon pouch life is significantly shorter than that of the tammar young and its growth rate so much greater (Rose 1989). Energy levels are high at pouch emergence and beyond, the very time that the young’s needs are greatest.

The growth of pouch young in this study was as described by Rose and McCartney (1982b). The relationship between the growth of the pouch young and the energy content of the milk is novel, if not surprising. One could surmise from this relationship that the amount of milk produced/consumed also increases linearly. Qualitative analysis of the carbohydrates in pademelon milk by thin layer chromatography (unpublished observations) showed that glucose, galactose and lactose were present in the milk and that these sugars were present at different stages of lactation.

The presence of relatively large amounts of carbohydrate (11–13% in the tammar and pademelon) presumably suit the nutritional requirements of the young up to mid-lactation; however, if this carbohydrate consisted only or mainly of lactose it could raise the osmotic pressure of the milk above that of plasma (Messer and Green 1979). Green and Renfree (1982) undertook an electrophoretic study of whey proteins in the tammar wallaby milk and found that the number and intensity of protein bands, particularly the α-globulin and pre-albumin bands, increased through lactation.

Table 2 lists the milk components and total solids during the three phases of lactation in the Macropodoidea (kangaroos, wallabies and rat-kangaroos). There are obvious differences between rat-kangaroo milk, on the one hand, and kangaroo and wallaby milks, on the other, with respect to the amount of protein in early lactation and the lipid levels in late lactation. Rat-kangaroos also produce a more concentrated milk, which may explain the much faster growth rates in the smaller members of this group (Rose 1989). These differences may be related to the differences in diet between the two families: rat-kangaroos are primarily fungivorous whereas the others are primarily herbivorous (grazing/browsing). The relationship between diet and milk composition is very interesting and well worth further study.

As a preliminary study in the qualitative aspects of whey proteins and carbohydrates, the results showed that milk in *Thylogale billardierii* generally exhibited similar trends, and is composed of similar constituents, to milk of other marsupials. It also showed that qualitative changes allow for the selection of milk that supplies the pouch young with its growth requirements.

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References


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