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Changes in Milk Composition during Lactation in the Potoroo, *Potorous tridactylus* (Marsupialia : Potoroinae)

H. M. Crowley[AC], *D. R. Woodward*[B] *and R. W. Rose*[A]

[A]Department of Zoology, University of Tasmania, G.P.O. Box 252C, Hobart, Tas. 7001.
[B]Department of Biochemistry, University of Tasmania, G.P.O. Box 252C, Hobart, Tas. 7001.
[C]Present address: Department of Zoology, Australian National University, G.P.O. Box 4, Canberra, A.C.T. 2601.

Abstract

Milk samples from captive potoroos were analysed for composition during weeks 3–25 of the lactation period. During pouch residence, up to week 16, carbohydrate levels were high, ranging from 9 g 100 ml^{-1} at week 5 to 15 g 100 ml^{-1} at week 15; fat levels were consistently low, at around 2 g 100 ml^{-1}; protein levels gradually increased from 5 g 100 ml^{-1} before week 10 to 12 g 100 ml^{-1} at week 16. Growth rates during this period increased exponentially, from 1 g $week^{-1}$ at week 3 to 40 g $week^{-1}$ at week 16. Thereafter, as the young left the pouch, marked changes were seen in carbohydrate and fat levels: by week 25, carbohydrate levels had fallen to 2 g 100 ml^{-1}, and fat levels had risen to 26 g 100 ml^{-1}. Protein levels increased moderately, reaching 15 g 100 ml^{-1} by week 25. Growth rates further increased during this period, to reach 60 g $week^{-1}$ by week 25.

Thus, trends in milk composition previously observed in *Macropus* species were observed also in the potoroo, suggesting a consistent pattern across the macropodid family. Carbohydrate levels in potoroo milk tend to be higher than in other macropodids, but total milk intake is as important as composition in determining growth rates.

Introduction

There is a large literature on eutherian milks, particularly from animals of economic or medical importance such as the cow, goat and man (Jenness and Sloan 1970; Oftedal 1984; Hartmann *et al.* 1985). Lactation plays an essential role in the reproductive cycle of the female marsupial; marsupials have a relatively short phase of intra-uterine growth and long period of lactation as compared with the eutherian. However, marsupial milks have, until recently, received relatively little attention, because of a lack of captive lactating marsupials and difficulty in obtaining adequate milk samples when the young are small.

Early work on the brushtail possum *Trichosurus vulpecula* (Gross and Bolliger 1958, 1959), opossum *Didelphis virginiana* (Bergman and Housley 1968) and red kangaroo *Macropus rufus* (Lemon and Barker 1967) showed that marsupials had a milk composition very different from that of eutherians. More detailed analysis of tammar wallaby (*Macropus eugenii*) milk has shown that milk composition changes markedly during lactation; this finding is common to all marsupials so far studied (Green 1984).

The potoroo, *Potorous tridactylus*, is a macropodid belonging to Potoroinae, a different sub-family from *Macropus*. It differs from larger macropods in that it breeds throughout the year and has a shorter pouch life (Hughes 1962; Guiler 1970; Shaw and Rose 1979). Unlike larger macropods, its diet is rich in fungi; its digestive physiology has unusual features, such as synthesis of essential amino acids from fungal precursors (Hume 1982; Frappell and Rose 1986).

The complete period of lactation in the potoroo, from initial attachment of the young to the teat until final weaning, is about 25 weeks. Although the young remains permanently attached to the teat for only 5–6 weeks, permanent pouch residence lasts approximately 17–18 weeks. The period of interim pouch vacation, which lasts from the first pouch exit to permanent

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pouch exit, is short, lasting about 10 days. From 19 weeks of age, the young animal lives permanently outside the pouch ('at foot').

The aim of the present study was to assess whether the trends in milk composition observed in *Macropus* species extended to another macropodid, *Potorous tridactylus.*

Materials and Methods

Collection of Milk

Milk was collected from lactating potoroos captured in the wild and held in captivity at the Zoology Animal House, University of Tasmania. In total, 54 samples from 16 females were collected from 3 weeks after birth to weaning at 25 weeks.

Young were removed from the teat for 3–5 h prior to milking. The mother was then injected with 0·15 ml oxytocin (Syntocin 10 i.u. ml^{-1}, Sandoz). Plastic tubing was placed over the teat, and milk was collected by suction into a 5 ml syringe attached to the other end of the tubing. The usual milk yield from this procedure was 0·4–1·0 ml. In the early stages of lactation, a general anaesthetic of 0·2 ml Ketamine (100 mg ml^{-1}) was also given to facilitate the collection of the milk. After milk collection, the young was replaced in the pouch.

Chemical Analyses

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). Thin-layer chromotography was used to monitor the qualitative changes in milk carbohydrates (Hansen 1975). Diluted whole-milk samples were applied to Silica Gel-60 plates in the solvent system, propan-2-ol/acetone/0·1 M lactic acid (4:4:2). Aniline-diphenylamine was used to stain the carbohydrate spots (Messer *et al.* 1984).

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, 89:11:3] 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 12 samples of potoroo milk, yielded a linear calibration equation: $F = (C - 2{\cdot}48)/1{\cdot}46$, where F is fat concentration (g 100 ml^{-1}), C is creamatocrit (%) and $r = 0{\cdot}96$. The fatty-acid composition of the lipids was assessed, in four milk samples, by gas chromatography (Green *et al.* 1983).

Sodium and potassium levels were determined by flame photometry.

Results

Total solids (Fig. 1*a*)

From weeks 5 to 25, the total solids increased from a minimum of 17 g 100 ml^{-1} at week 4 to a maximum of 53 g 100 ml^{-1} at week 21. The increase was initially slow and steady, but after week 15 it became more rapid and subject to greater fluctuations.

Protein (Fig. 1*b*)

Up till week 10, the total protein level remained fairly constant at 5 g 100 ml^{-1}. Thereafter, it showed a steady increase, reaching 12 g 100 ml^{-1} by weeks 16–17, and 15 g 100 ml^{-1} at week 25.

Carbohydrate (Fig. 1*c*)

Total carbohydrate concentration showed a moderate, but erratic, increase during pouch residence, from *c.* 9 g 100 ml^{-1} at week 5 to *c.* 15 g 100 ml^{-1} at week 15. Levels then decreased sharply, reaching 2 g 100 ml^{-1} at week 25.

Thin-layer chromatography revealed major qualitive changes in milk carbohydrates. Up to week 15, most of the carbohydrate consisted of slow-moving spots (presumably oligosaccharides) with small amounts of material corresponding to lactose, galactose and glucose (Fig. 2). Thereafter, the oligosaccharide spots became progressively less intense, and the mono- and disaccharide spots more intense.

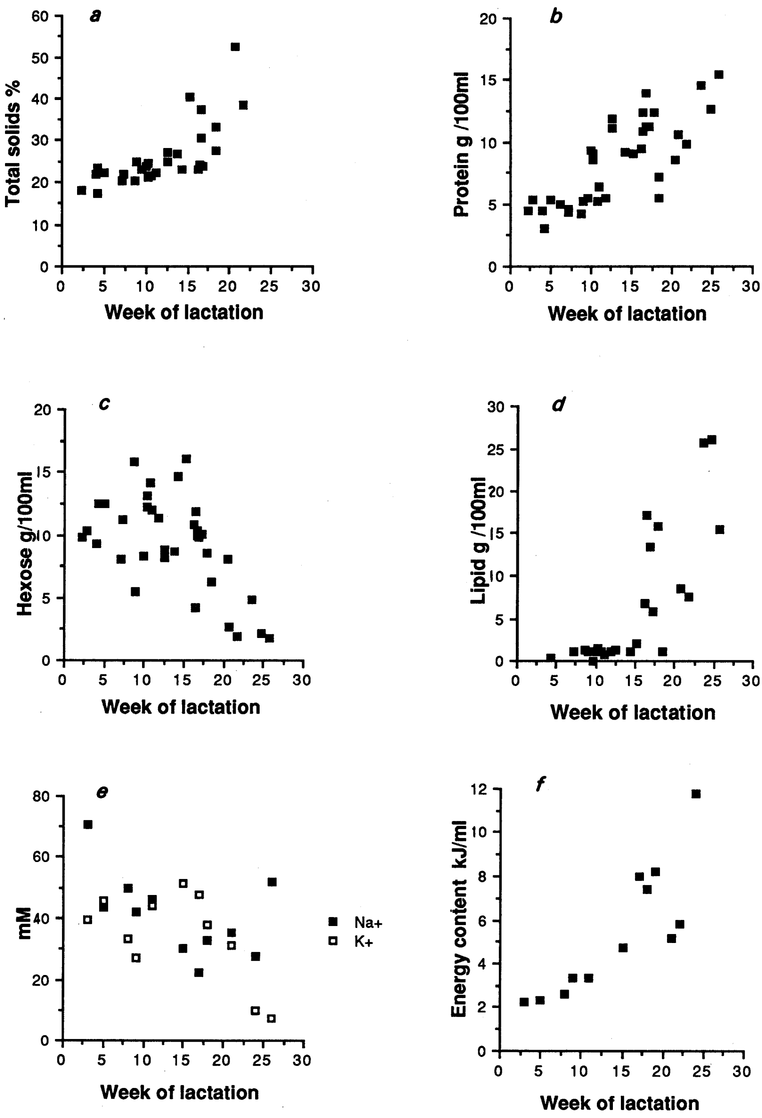

Fig. 1. Concentration of various constituents of potoroo milk during lactation. Each point represents one sample.

A milk sample from week 10 was hydrolysed at 100°C (2 M HCl, 1 h). Thin-layer chromatography showed absence of the slow-moving spots, and intense glucose and galactose spots (Fig. 3). This result confirmed that the slow spots were probably glucose–galactose oligosaccharides.

Lipid (Fig. 1*d*)

Total lipid concentration of milk remained low until week 15 at around 2 g 100 ml^{-1}, but showed a marked increase thereafter, reaching 26 g 100 ml^{-1} by week 25.

The fatty-acid composition of the triglyceride fraction was determined for four samples, taken at 12, 16, 18 and 20 weeks, respectively. The predominant fatty acids were oleic acid (C18:1), palmitic acid (C16:0) and linoleic acid (C18:2). Stearic acid (C18:0) and palmitoleic

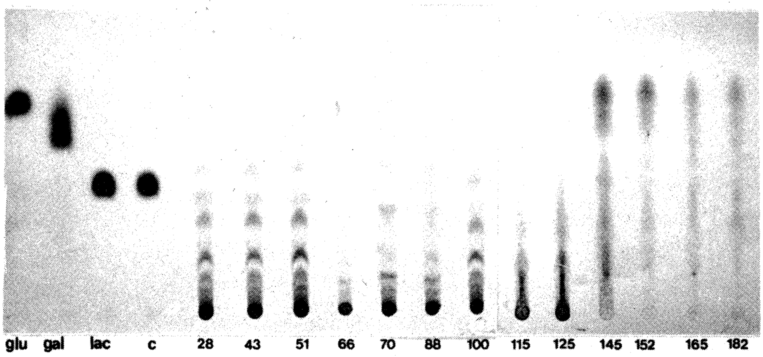

Fig. 2. Thin-layer chromatography of carbohydrates of potoroo milk during lactation; the numbers indicate days since start of lactation. Also included is a sample of cows' milk (c).

acid (C16:2) were also found in significant quantities in all samples (Table 1) but, notably, short-chain fatty acids were not found.

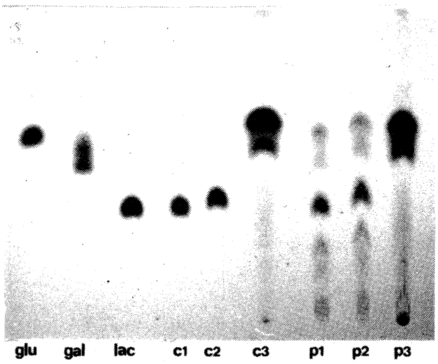

Fig. 3. Effect of acid hydrolysis on milk carbohydrates from cow (mature milk) and potoroo (week 10). Samples are denoted c (cow) and p (potoroo): those labelled 1 are untreated milk; 2, milk mixed with HCl, but not heated; 3, milk heated with HCl to hydrolyse glycosidic links.

Table 1. Fatty acid profile of potoroo milk
Expressed as mol of each fatty acid per 100 mol total fatty acids

Fatty acid	Stage of lactation (weeks)			
	12	16	18	20
C14:0	1·40	3·04	2·94	2·55
C14:1	0·08	0·07	0·18	0·43
C15:0	1·33	1·08	1·06	0·52
C15:1	0·09	0·08	0·08	0·09
C16:0	27·86	33·55	27·26	29·00
C16:1	7·05	6·19	8·01	11·81
C17:0	1·73	1·38	1·43	1·05
C17:1	0·42	0·55	0·68	0·65
C18:0	3·96	6·75	5·12	4·26
C18:1	38·94	30·09	35·80	37·18
C18:2	13·73	12·96	13·62	10·07
C18:3	1·25	1·24	1·52	0·95

Sodium and Potassium (Fig. 1*e*)

Levels of both these electrolytes were rather erratic throughout lactation. The general trend was for decreasing levels of both; however, after week 22, sodium appeared to increase as potassium decreased.

Energy (Fig. 1*f*)

Using the conventional (Thomas and Corden 1977) values of 16 kJ g^{-1} (carbohydrate), 17 kJ g^{-1} (protein) and 37 kJ g^{-1} (fat), the average energy content of milk was estimated at various times during lactation. A steady increase occurred up to week 11, with a rapid increase thereafter.

The proportions of energy derived from the different milk constituents showed dramatic shifts (Fig. 4). During weeks 3–15, protein contributed about one-third of the energy; this fell

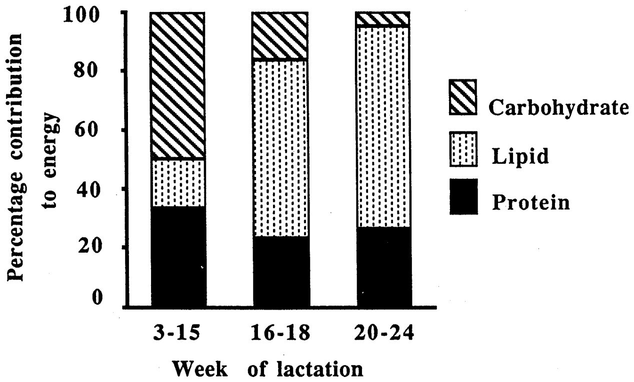

Fig. 4. Mean contribution of protein, carbohydrate and lipid to total energy content of potoroo milk, at different stages of lactation.

to 20–25% during weeks 16–24. Carbohydrate contributed about half the energy during weeks 3–15, about 20% during weeks 16–18, and less than 10% during weeks 20–24. Lipid contributed only about one-tenth of energy during weeks 3–15, but 60–65% of energy thereafter.

Growth rate of the young (Fig. 5)

Initially, weight gains were low in absolute terms, though high in relation to body weight. From about 11 weeks, there was marked increase, to a plateau from around 15 weeks. (Results have been extended past week 15 with unpublished data from Bryant and Rose.)

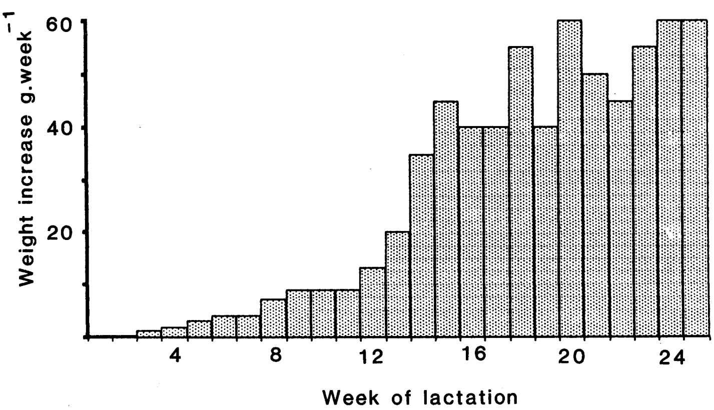

Fig. 5. Absolute growth rate of potoroo young.

Discussion

Marsupial Milk Composition over the Lactation Cycle

In marsupials, the milk shows a continuous change in composition during lactation which is apparently related to the growth and increasing independence of the young. It is therefore unfortunate that not all published analyses of marsupial milks have indicated the stage of lactation at which the sample was taken.

Methodological differences also hamper comparisons of published values. This problem is particularly acute for carbohydrate assays. While it was long suspected that the carbohydrate in marsupial milks was not lactose (Jackson and Rothera 1914; Gross and Bolliger 1958; Jenness *et al.* 1964) it was only recently established that oligosaccharides predominate in the pouch-residence phase, with small amounts of free monosaccharides present throughout lactation (Messer and Mossop 1977; Messer and Green 1979). This was confirmed for the potoroo by chromatography of the carbohydrates (Fig. 2). Based on the assumption that lactose was the major milk sugar, almost all published reports yield misleading results. The method used in this paper gives a good estimate of the total carbohydrate, although it may underestimate any amino sugars present (Messer and Green 1979).

The most complete set of milk composition data is for the tammar wallaby *Macropus eugenii*: detailed reports have been published on tammar milk carbohydrates (Messer and Mossop 1977; Messer and Green 1979), fats (Green *et al.* 1983), proteins (Green *et al.* 1980; Green and Renfree 1982) and electrolytes (Green *et al.* 1980). Less extensive composition data have been reported for the red kangaroo *M. rufus* (Lemon and Barker 1967; Griffiths *et al.* 1972; Poole *et al.* 1982) and the grey kangaroo *M. giganteus* and *M. fuliginosus* (Poole *et al.* 1982).

Changes in milk composition over the lactation cycle show similar trends in all the *Macropus* species studied. The potoroo data presented in this paper (Fig. 6) show the same trends, as

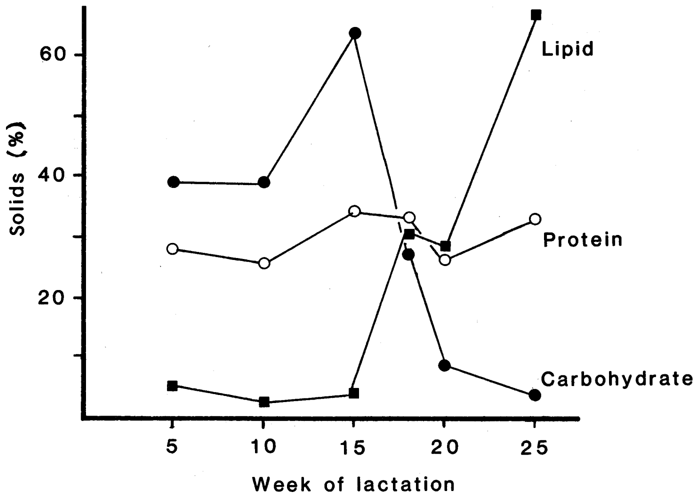

Fig. 6. Mean contribution of protein, carbohydrate and lipid to total solids content of potoroo milk, at different stages of lactation.

do data (Smolenski and Rose 1988) for another potoroine macropodid, the Tasmanian bettong *Bettongia gaimardi*. There is thus remarkable similarity of milk compositional changes in both macropodid sub-families.

In absolute values, potoroo milk shows differences from *Macropus* milk at comparable stages of lactation. It has lower fat levels (perhaps due to methodological differences), and higher levels of carbohydrate and electrolytes.

Growth Rate in Relation to Milk Composition and Consumption

Potoroo pouch young have one of the highest relative rates of growth among the macropods, second only to their near relatives the bettongs (Rose, unpublished data). The finding of relatively high levels of carbohydrate in potoroo milk throughout pouch residence is consistent with their high growth rates.

Growth rates of pouch young of other macropod species appear to be limited by the supply of milk from the mother (Green 1984). Experiments involving exchange of pouch young of

Wallabia bicolor and *Macropus rufus* between mothers at different stages of lactation or mothers of different species showed that the normal growth of the pouch young is not necessarily maximal (Merchant and Sharman 1966). Similar results have been reported for intra-species exchanges in the Tasmanian bettong (Rose 1986).

This highlights two important points. First, it is critical to study the amount of milk consumed by the young, as well as the quality of milk, before conclusions on growth energetics can be made. Secondly, milk composition and consumption are not only affected by the demands of the young, but also represent a compromise between the need to conserve maternal resources, the availability of resources in the environment and the requirements of the young (Oftedal 1980). The absolute growth rate of potoroos (Fig. 5) and parma wallabies (Maynes 1976) reaches a plateau around the time of pouch vacation, followed by a rise after pouch exit. These data suggest that, despite the increased energy content of the milk, total milk energy relative to the energy requirements of the young becomes limiting towards the end of pouch residence; this may be one factor initiating pouch emergence (Rose 1986).

This study has shown that, in the potoroo, as in other marsupials, major changes take place in the milk composition around the time of pouch vacation. Major changes also occur in the young at this time. The increased activity and development of endothermy result in a higher energy demand, which in turn is reflected in the dramatic increase in milk fat and energy levels (Rose 1987). There are metabolic changes, and structural and functional changes in the gastro-intestinal tract (Walcott and Messer 1980; Paton and Janssens 1981; Vernon *et al.* 1981). In the potoroo, these changes do not result in adult digestive efficiency until well after pouch vacation. Evidence for this is the continuing functioning of the gastric sulcus and the low numbers of gut microflora (Frappell 1984). This suggests that milk, in addition to being vital to the young throughout pouch life, is also an important dietary and energy supplement during pouch vacation and weaning as the young adapts to solid-food intake.

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