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Milk Composition in the Eastern Quoll, *Dasyurus viverrinus* (Marsupialia : Dasyuridae)

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Abstract

The milk constituents of *Dasyurus viverrinus*, a carnivorous marsupial, exhibited major quantitative and qualitative changes during the course of lactation. The milk produced in the early stages of lactation was dilute, about 13–16% (w/w) solids before 3 weeks with carbohydrate representing the major fraction. In the latter stages of lactation the milk was concentrated, around 30% solids, and lipid was the predominant fraction. Palmitic acid was the major fatty acid present in early-stage milk but oleic acid became predominant in milk after 10 weeks post-partum. The changes in milk composition in *D. viverrinus* were similar to those described for the milks of herbivorous marsupials which therefore suggests that this pattern may be uniform throughout the Marsupialia.

Introduction

Milk composition has been analysed in a number of marsupials, but species that have been most studied are monotocous macropodids — wallabies and kangaroos (Green 1984). These herbivorous species exhibit major qualitative and qantitative changes in milk composition throughout lactation. The early milk is very dilute and consists predominantly of hexose, in the form of oligosaccharides. Lipid levels, in which palmitic acid (C16:0) is the major fatty acid, are low. Late-stage milk is very concentrated and is characterized by low hexose levels, in the form of monosaccharides, and high lipid levels in which oleic acid (C18:1) predominates.

This study was undertaken to determine whether milk composition in a carnivorous, polytocous marsupial, *Dasyurus viverrinus*, was similar or substantially different to that described for the herbivorous, monotocous macropodids.

Materials and Methods

Animal Colony

The general maintenance of the animals used in this study has been described in detail by Merchant *et al.* (1984). Lactating females were individually housed with their offspring in pens 1.8 by 0.9 m and 1.8 m high, either outside in natural daylight and exposed to ambient temperatures, or in an air-conditioned animal house at 18°C and a 12 h light : 12 h dark regime. The breeding season of the eastern quoll commences towards the end of May and births occur 18–20 days later in mid-June. Births are usually restricted to a period of about a week so that females in this study were highly synchronized, all having young of similar age.

During the study a total of 21 females each provided one or more sequential milk samples per lactation. Ten females provided 1–5 samples, five provided 6–10, four provided 11–15 and two females provided 21 and 23 samples. Not all samples were sufficient in quantity for all analyses. Therefore, total milk solids were based on 109 samples from 16 females; electrolytes on 97 samples from 12 females; carbohydrates on 131 samples from 21 females; protein on 53 samples from six females and crude lipid on 47 samples from six females.

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Sixteen of the 21 females used in this study also provided data for the paper on growth of the young eastern quoll (Merchant *et al.* 1984). No evidence was available in that paper to suggest that young in litters of different size grew at different rates. There is therefore no evidence in the present study to suggest that the quality or quantity of milk available to an individual young differs from one lactation to another.

Milk Collection

During the first 6 weeks of lactation milk samples could only be obtained by killing the young, as it was impossible to reattach young to the teats. From about 7 weeks of age young were removed from the teats and placed in a humid incubator at 35°C. From about 9 weeks of age, when the pelage was well developed, they were kept warm and dry in small calico bags. Milk was allowed to accumulate in the mammary glands for about 5 h, after which mothers were anaesthetized by intravenous injections of sodium methohexitone (Brietal Sodium, Eli Lilley and Co. Sydney; 12 mg/kg body weight) into a marginal ear vein. When the young were about 17 weeks old it was found that the mothers were so amenable to handling that anaesthesia was unnecessary. In the second year of the study, mothers were milked without anaesthesia from about 7 weeks post-partum. The mothers were restrained in a calico bag and the mammary glands exposed for milking. The teats were wiped clean with a tissue and an intramuscular injection of oxytocin (Syntocinon; Sandoz, Australia) was given at a dosage of 1 i.u./kg body weight. Milk started to appear at the tips of the teats after 1 or 2 min, and was collected either by pipette or directly into small plastic vials. Without the administration of oxytocin it was found to be very difficult to obtain sufficient quantities of milk for some analyses.

Milk was collected at intervals of between 7 and 12 days. Later in the study four females were milked more frequently during specific periods when major changes in milk composition were taking place. These latter females were milked twice weekly from 15 weeks through to the end of lactation.

During milk collection it was noticed that a clear fluid was often expressed with the first milk. This first milk was found to have uncharacteristically high electrolyte concentrations so that it became standard practice to discard the first 50 μl approximately of milk and use only the subsequent milk for analysis. Young which had been removed from the teat to allow milk accumulation were assisted back on to the teat up to about 60 days and thereafter were able to grasp the teat easily when placed against it. Milk samples from individual teats of the same female were pooled for subsequent use in milk analyses. The earliest milk samples were obtained from five lactating females in the field. The volumes obtained were small so that samples were pooled to allow estimation of total solids and carbohydrate (hexose). The estimated ages of the young of these females were 21, 20, 18, 17 and 15 days. Three of these females provided sufficient milk to enable individual determinations for sodium and potassium concentrations to be made.

Milk Analyses

Total solids

Depending on the availability of milk, volumes of between 30 and 100 μl approximately, were weighed in preweighed plastic vials. Each sample was then freeze-dried and reweighed to register total solids. All weighings were made to the nearest 0.1 mg on a Mettler balance (model KST).

Sodium and potassium

Sodium and potassium estimations were carried out using the method of Green *et al.* (1980) except that 5 μl of milk rather than 10 μl was used. The electrolyte concentrations are expressed as millimoles per litre of milk (mmol/l).

Carbohydrate

Total hexose was estimated by diluting 5 μl of milk with distilled water and then assaying 200 μl aliquots with a modified phenol-sulfuric acid reagent (Messer and Green 1979).

Lipid

Crude lipid, the triglyceride fraction of the lipid and the fatty acid complement of the crude lipid and triglyceride fraction were determined by the methods of Green *et al.* (1983). The total lipid of milk samples obtained in the first 10 weeks of lactation were estimated as the difference between measurements of protein, hexose and total solids, assuming an ash content of 5% of the solids fraction.

Total nitrogen

The dried residues from the total solid determinations were digested using the Kjeldahl procedure. Total nitrogen was estimated by a microdiffusion technique (Conway 1962) and converted to protein using a factor of 6.38.

Results

Milk Solids

The composition of the milk changed considerably throughout lactation. At 3 weeks into lactation about 16% (w/w) was solid material but by about 15 weeks, when the young first began to eat solids, this had risen to just over 30% (w/w) (Fig. 1).

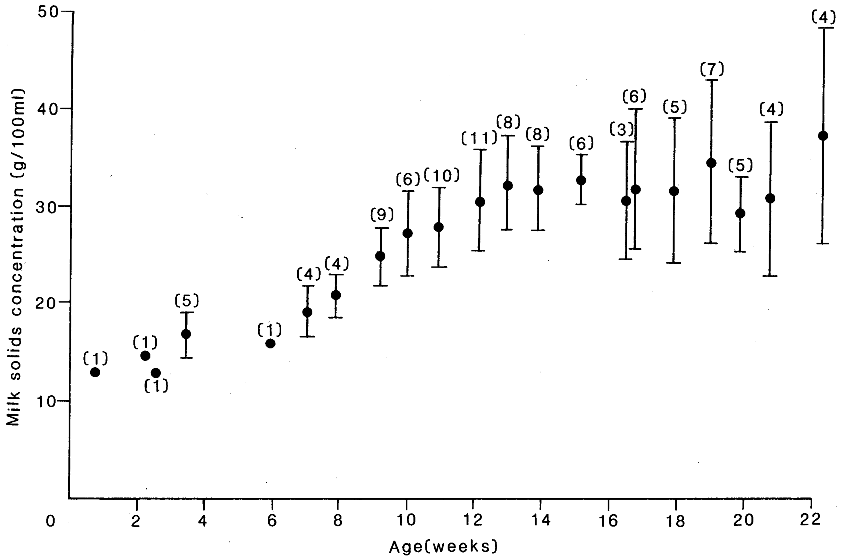

Fig. 1. Changes in total milk solids during lactation. Values are given as means ± 1 s.d. (*N*).

From 15 weeks to about 20 weeks the solid constituents remained fairly constant and by 22 weeks (Merchant *et al.* 1984) some young were totally weaned. The results after about 20 weeks became increasingly variable in those animals which continued to lactate. These were females with larger litters (Merchant *et al.* 1984) and it is felt that the prolonged lactation of these animals may have been induced by conditions of relatively close confinement in captivity. In the field, few animals are found lactating after early November so that 20–22 weeks may be regarded as the more usual period of lactation. For the purposes of this study the results to 22 weeks are considered to be indicative of the natural situation.

Electrolytes

Electrolyte levels in the milk changed during lactation with sodium concentrations initially higher, about 45 mmol/l, than potassium, about 30 mmol/l (Fig. 2). At about 9–10 weeks into lactation the sodium and potassium curves crossed so that by 11 weeks potassium reached maximum values of 50–55 mmol/l while sodium was then around 30 mmol/l.

Subsequently both electrolytes decreased in concentration, potassium to about 40 mmol/l and sodium to 20 mmol/l at 20 weeks. Although results after this time are sparse they suggest a trend towards plasma concentrations, with potassium declining to 25 mmol/l, one individual as low as 10 mmol/l, and sodium increasing towards 30 mmol/l with one individual reaching 115 mmol/l.

Carbohydrates

Carbohydrate concentrations of between 3 and 5 g/100 ml were measured in the first 4 weeks of lactation but then increased to maximum values of 7.4 g/100 ml by 8 weeks post-partum (Fig. 3*a*). Between 10 and 17 weeks hexose concentrations were uniform at about 5 g/100 ml after which they declined to around 2 g/100 ml at 22 weeks. Values as low as 0.70 g/100 ml were recorded as early as 21 weeks in some females, but were more usual in the period after 22 weeks post-partum.

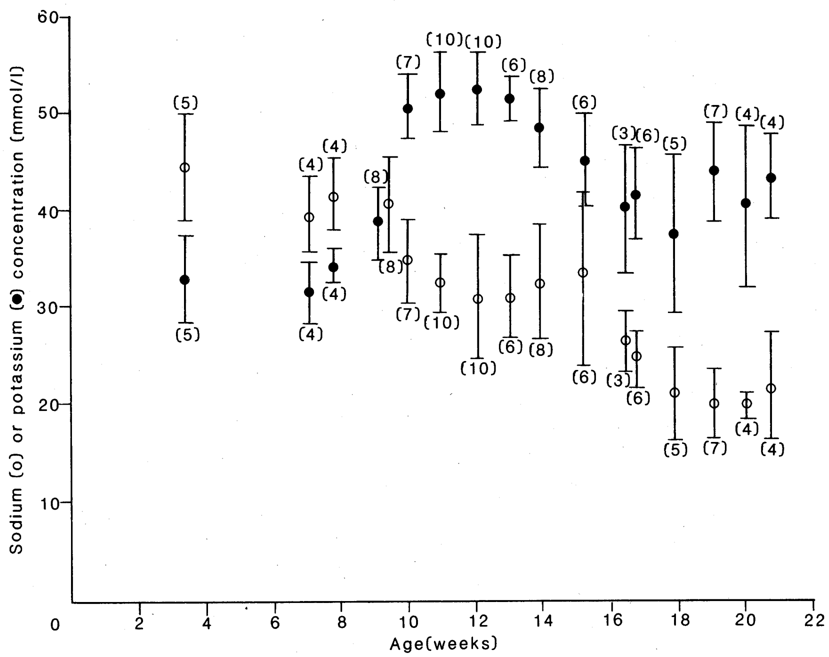

Fig. 2. Changes in sodium and potassium concentrations in milk during lactation. Values are given as means ± 1 s.d. (*N*).

Protein

The nitrogen content of the milk, expressed as protein, is shown in Fig. 3*b*. The data for early lactation are sparse but suggest a rise from about 3% (w/w) at 3 weeks to about 8.6% (w/w) at 9 weeks post-partum. Thereafter mean values ranged from 7.5 to 10% (w/w).

Lipid

The crude lipid content of the milk was around 4% (w/w) at 7 weeks and was followed by a gradual but consistent increase to 15% (w/w) at 15 weeks. Thereafter levels were maintained at this concentration (Fig. 3*c*), except for some exceptional values between 25 and 33% (w/w) in one female.

The predominant fatty acids of the milk triglycerides are shown in Table 1. From 12 weeks post-partum through to the end of lactation oleic acid (C18:1) represented almost 40% (w/w) of the milk triglycerides, while palmitic acid (C16:0) represented about 25% (w/w). Only small amounts of pooled milk were available for analyses prior to 12 weeks post-partum, but these indicate that palmitic acid predominates in early milk, with a crossover between palmitic and oleic acid levels occurring between 6 and 10 weeks post-partum.

Carbohydrate : Lipid : Protein Ratios

The relative contributions of carbohydrate, lipid and protein to the solids of the milk has been calculated from the preceding data and is presented in Fig. 4. During the first 7 weeks of lactation carbohydrates constitute around 40% of the solids fraction while lipid is about 20% and protein about 30%. Between 7 and 9 weeks, carbohydrate begins to decline while

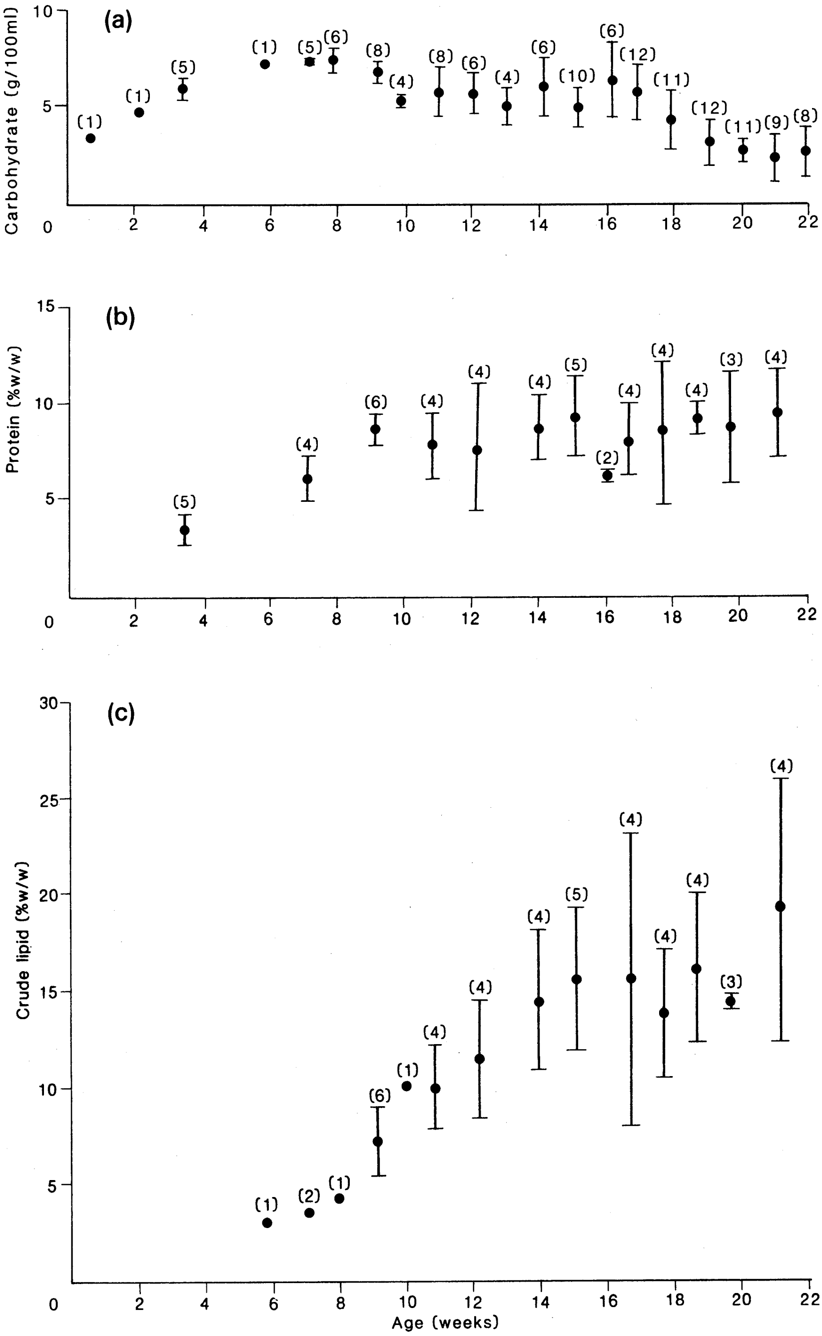

Fig. 3. Changes in carbohydrate (*a*), protein (*b*) and crude lipid (*c*) concentrations during lactation. Values are given as means ± 1 s.d. (*N*).

lipids increase so that both reach about 30%, a similar value to that for protein. The respective decline and increase of carbohydrate and lipid continues so that by 22 weeks carbohydrate represents about 7% and lipid 50% of the milk solids. Protein is still about 30% at this stage.

Table 1. Major fatty acids of the milk triglycerides
Values are mean percentages (w/w) of the total triglyceride complement ± 1 s.d.

Age of young (weeks)	No. of animals	Fatty acid 14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
6	P[A]	2.55	46.79	7.52	3.84	23.52	11.96	0.85	Trace
	1	2.16	39.22	6.93	7.85	32.61	5.76	Trace	Trace
10	4	1.22	26.98	4.47	7.15	33.79	16.68	1.31	1.25
		±0.39	±4.80	±1.78	±1.92	±4.53	±1.69	±0.25	±0.31
12	3	1.49	27.65	5.08	6.23	36.19	16.29	1.20	1.41
		±0.28	±4.07	±1.37	±1.72	±4.08	±1.58	±0.54	±0.20
14	3	1.65	24.61	4.04	8.70	36.10	15.37	2.20	1.56
		±0.18	±0.74	±0.22	±0.76	±1.26	±1.01	±0.23	±0.22
19	3	1.95	22.27	5.51	7.01	36.89	15.01	2.77	1.27
		±0.57	±0.79	±0.52	±0.96	±1.41	±1.77	±0.83	±0.20
22	4	2.07	21.88	8.72	4.95	37.05	16.30	2.31	1.40
		±0.39	±1.10	±1.26	±0.48	±2.95	±0.98	±0.27	±0.34

[A]P, pooled sample.

Discussion

The gradual but major increase in the concentration of milk solids that takes place during lactation in *D. viverrinus* is similar to the pattern that has been shown for tammar wallabies, *Macropus eugenii* (Green *et al.* 1980) and other marsupials that have been studied so far (reviewed by Green 1984).

The maximum concentration of solids in the final stages of lactation in *D. viverrinus*, 30% (w/w), is lower than the 40% solids found in the late stages of lactation in tammar wallabies (Green *et al.* 1980) and brush-tailed possums, *Trichosurus vulpecula* (Gross and Bolliger 1959) but higher than the 23% solids fraction of the red kangaroos, *Macropus rufus* (Lemon and Barker 1967).

The increase in milk solids during lactation in marsupials probably coincides with changes in the suckling regime of the young. In the early stages of lactation young marsupials are continuously attached to the teats and in some species, e.g. *D. viverrinus*, the young are so firmly attached that removal from the teat results in buccal damage and the young cannot be replaced on the teat. It is likely that the young suckle frequently during this period of close attachment.

In later stages of lactation the young can relinquish the teats and, in the case of *D. viverrinus*, are deposited in a den at about 9 weeks post-partum. Suckling bouts thereafter are probably progressively less frequent and coincide with the production of concentrated milk. A correlation between the concentration of milk solids and suckling frequencies in mammals has been demonstrated by Ben Shaul (1963) and Jenness and Sloan (1970).

The rise in the milk hexose concentrations during the first 8 weeks of lactation in *D. viverrinus* is similar to that which occurs in *M. eugenii* during the first 26 weeks of lactation (Messer and Green 1979), but the peak values of 7% in *D. viverrinus* are about half those found in *M. eugenii*. After this time there is a precipitous decline in milk hexose in *M. eugenii* accompanied by a change from oligosaccharides to monosaccharides. However, there is a more gradual decline in milk hexose in *D. viverrinus*, but again it is associated with a change from oligo- to monosaccharides (Messer *et al.* 1987).

The crude lipid levels in *D. viverrinus* milk reflect the general increase in milk solids during lactation although a steep increase in lipid concentration is apparent between 9 and 15 weeks post-partum. Again this pattern is similar to that found in *M. eugenii* and other marsupials (Green 1984). The pattern of changing levels of fatty acids in the triglyceride fraction of *D. viverrinus* milk is also similar to that in other marsupials, with palmitic acid predominating in the first 6 weeks or so post-partum and oleic acid predominating thereafter (Griffiths *et al.* 1972; Green *et al.* 1983).

Two other major fatty acids in *D. viverrinus* milk are stearic acid (C18:0) and linoleic acid (C18:2) which are present to the extent of about 7 and 15% (w/w) respectively of the triglyceride fraction. In comparison these two fatty acids are present at about 18 and 7% (w/w) respectively in *M. eugenii* milk (Green *et al.* 1983).

Arachidonic acid (C20:4) was found in *D. viverrinus* milk to a much greater extent than in *M. eugenii* and probably reflects the insectivorous diet of *D. viverrinus*.

The protein content of the milk increased from about 3% in the early stages of lactation to a peak of about 8% in later stages. These values are lower than those recorded for *M. eugenii* (Green *et al.* 1980; Green and Renfree 1982), *T. vulpecula* (Gross and Bolliger 1959) and *Didelphis virginiana* (Berman and Housley 1968) but similar to *M. rufus* (Lemon and Barker 1967).

The pattern of changes in sodium and potassium concentration in *D. viverrinus* milk is similar to that found in two macropodid marsupials, *M. eugenii* and *Setonix brachyurus* (Green *et al.* 1980; Bentley and Shield 1962). The sodium concentration of *D. viverrinus* milk is higher than potassium for the first half of lactation, after which potassium concentrations exceed those of sodium. The crossover point for sodium and potassium concentrations in *D. viverrinus* at about 9 weeks coincides with teat release by the pouch young (Merchant *et al.* 1984) and

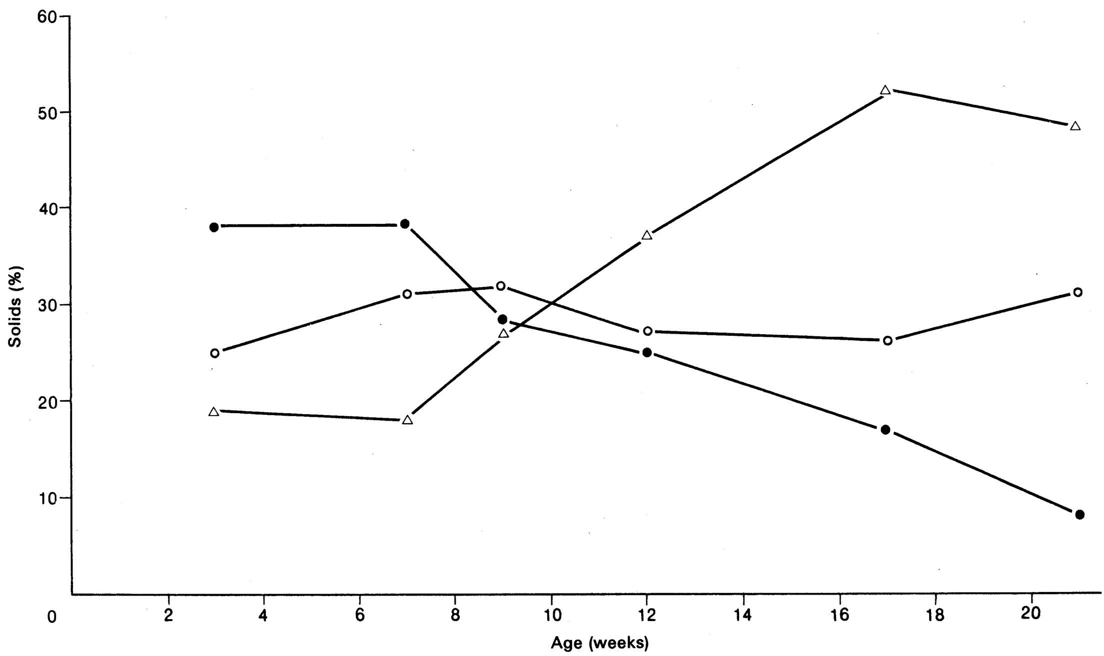

Fig. 4. Relative proportions of carbohydrate (●), lipid (Δ) and protein (○) in the milk of eastern quolls throughout lactation expressed as a percentage of the solids fraction (w/w).

their deposition in a den. The crossover in *M. eugenii* and *S. brachyurus* similarly coincides with teat release by the pouch young of both species. The termination of lactation is marked by electrolyte levels in milk approaching those of serum, presumably as a result of breakdown in the tight junctions of the secretory epithelia.

The pouch exit of *D. viverrinus* young and their deposition in a den also coincides with the major changes in concentration of lipid and carbohydrate in milk. The lipid-carbohydrate crossover in *M. eugenii* occurs relatively later in lactation but again coincides with pouch vacation. It is also of interest that the major changes in milk composition occurring at pouch vacation coincide with a marked increase in circulating prolactin levels, from around 40 ng/ml to 160 ng/ml (Hinds and Merchant 1986). These authors suggest that the increased prolactin levels are associated with a changed suckling pattern; fewer suckling episodes but of greater intensity and/or duration.

The changes in milk composition found in *D. viverrinus* are generally similar to those described for other, predominantly macropodid, marsupials (Green 1984). It therefore appears that marsupials, in general, demonstrate a similar pattern of quantitative and qualitative changes in milk composition throughout lactation. However, the precise nature of the relationship between changes in milk components and the developmental stages of marsupial pouch young are still unclear.

Acknowledgments

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