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Composition of Milk from Red and Grey Kangaroos with Particular Reference to Vitamins

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Abstract

Analysis of milk from red and grey kangaroos confirmed that the composition of milk from individual mammary glands changes with advancing age of the associated suckled young. Although the proportion of particular components may change, concentration of solids, in particular fats and to a lesser extent protein and casein, increase throughout lactation. The results of analyses to determine the vitamin content of kangaroo milks are presented for the first time.

Introduction

Because of their disjunctive geographic distribution and their long history of independent evolutionary development, marsupials have always aroused great interest in any study of comparative zoology. In particular, their characteristic mode of reproduction, combining a short gestation with the production of a small and relatively undeveloped neonate to be nurtured by the female parent in an integumentary abdominal pouch, has provided ready access for studies of 'foetal' growth. Growth of the young from such an early age is solely dependent on nourishment obtained from milk. At times in kangaroos two young, separated in age by at least 6 months and presumably with different nutritional demands, may be suckling individual teats in the same pouch. This has prompted investigations of the composition of milk from a variety of marsupial species.

Early studies indicated that the concentration of total solids, fats, protein, ash and sugars were higher in marsupials' milk than in cows' milk and that these concentrations changed as the pouch young grew older (Bolliger and Pascoe 1953; Gross and Bolliger 1959; Bolliger and Gross 1960). More recent investigations have examined aspects of diverse constituents in milks from a wider range of marsupial species. For example, from among the subfamily Macropodinae, amino acids have been investigated by Renfree *et al.* (1982); proteins by Jordan and Morgan (1968), Bell *et al.* (1980) and Green *et al.* (1980); carbohydrates by Messer and Mossop (1977) and Messer and Green (1979); and lipids by Glass *et al.* (1967), Griffiths *et al.* (1972) and Grigor (1980). Several of these studies together with that undertaken by Lemon and Barker (1967) have followed both qualitative and quantitative changes during lactation and, in addition, Lemon and Bailey (1966) have confirmed qualitative differences in the whey proteins of milk drawn from separate glands of the one animal.

This paper presents the results of analysis of milk from the red kangaroo, *Macropus rufus*, and the grey kangaroos, *M. giganteus* and *M. fuliginosus*. Apart from additional information on major constituents of kangaroo milk, the concentrations of the component vitamins in marsupial milk are provided for the first time.

Methods

Collection of Milk Samples

In 1964 and 1965 milk was obtained from captive red kangaroos and in 1964 and 1970 from grey kangaroos carrying young of known age and held in outdoor enclosures at the Division of Wildlife Research, CSIRO. The young were removed from the pouch while the milk was collected. Their mothers were anaesthetized for a period of 20–30 min following an intravenous injection, via one of the lateral caudal veins, of a 2·5% solution of methohexitone sodium (Brietal sodium: Eli Lilly) at a rate of 7·5 mg/kg. Immediately through the same hypodermic needle, 5 i.u. oxytocin (Pitocin: Parke-Davis) was administered to assist in the let-down of milk. The elongated lactating teat and associated mammary gland were washed with a 0·1% aqueous solution of cetrimide (Cetavlon: ICI) then the milk stripped by hand directly into a 25 ml Macartney bottle.

Milk was also collected from a small number of red kangaroos shot in 1965 on Toganmain Station, near Hay, New South Wales. Oxytocin was administered intramuscularly immediately each animal was shot, then a sample of milk was stripped from the teat.

In order to provide a sufficient volume of milk for analysis, it was necessary in several cases to combine samples of milk drawn from a number of animals at a similar stage of lactation. The aim was to collect the required volume of milk in the shortest possible time, in order to minimize deterioration, rather than to collect sequential samples throughout lactation. No measure of the total volume of milk secreted was attempted. A subsample to which a buffer was added (see Ford 1967) to stabilize the milk folate, was held separately for the folic acid assay. The samples of milk were protected from light and held frozen until shortly after collection when they were packed in dry ice and airfreighted to the National Institute for Research in Dairying, Shinfield, England, where they were kept at −25°C. All the analyses of each shipment were completed within 9 months of receipt.

Analysis of Milk Samples

The fat content of the milk was measured by the Rose–Gottlieb method and the fatty acid composition of the milk fat by gas–liquid chromatography. Total solids were determined by drying at 100°C and total protein and casein by the Kjeldahl method, the casein having been separated as described by Rowland (1938). Ash was measured by incineration at 500°C. Calcium was measured as the oxalate and phosphorus as ammonium phosphomolybdate.

Vitamins of the B-complex were assayed microbiologically. The procedures used for riboflavin, nicotinic acid and biotin were those described by Ford *et al.* (1953) and Chapman *et al.* (1957). Folate activity was assayed with *Lactobacillus casei* as described by Ford (1967). Vitamin B_6 was assayed with *Kloeckera apiculata* (*brevis*) as described by Barton-Wright (1963) except that the samples were extracted with 0·05 M HCl as recommended by Gregory (1959). Vitamin B_{12} was assayed with *Lactobacillus leichmannii* as described by Gregory (1954). Thiamine was assayed with *Lactobacillus fermentum* 36; the test medium was that of Banhidi (1958) and the samples were extracted by heating for 30 min at 100°C with 0·016 M H_2SO_4.

For the determination of retinol, α-tocopherol and active carotenes, samples of the milk or, in some instances, of the separated cream were diluted with warm distilled water. The fat was then extracted as described by Olsen *et al.* (1939) and weighed. Most samples provided less than 2·5 g fat for analysis and this was treated as follows. The fat was saponified by heating for 10 min on a steam-bath with 5 ml ethanol (A.R.), 1·1 ml KOH solution (60% w/w) and 50 mg sodium ascorbate. Following the addition of 20 ml distilled water, the whole was extracted three times with 20-ml portions of diethyl ether (A.R.). The extracts were then combined and washed three times with 20-ml portions of water. The solvent extracts were not dried with anhydrous sodium sulfate, as this procedure could have introduced traces of iron that would interfere in the estimation of tocopherols.

For the estimation of retinol and carotene, a sample of the ether extract was evaporated and the residue dissolved in n-hexane (boiling range 67–69°C) and chromatographed on aluminium oxide (BDH, Brockmann grade 11) as described by Thompson *et al.* (1949). The carotenes were determined spectrophotometrically (Thompson, 1949) and retinol from the intensity of the blue colour formed on the addition of a solution of antimony trichloride in chloroform. Crystalline retinol was employed as the calibration standard (Fisher *et al.* 1956).

The procedure of the Analytical Methods Committee (Anon. 1959) was used to measure α-tocopherol, but was modified in that purification on Floridin Earth XS was replaced by the simpler procedure of employing Decalso F, as described by Crane *et al.* (1959).

Table 1. Details of milk samples from red and grey kangaroos and their major components at different stages of lactation

— Insufficient sample available to undertake analyses

Age (days)	No. of donors	Total milk (ml)	Total solids	Fat	Total protein	Casein	Ash	Ca	P
			← (expressed as g/kg milk)						→
			Red kangaroo						
Pouch young									
11	1	1·5	—	—	—	—	—	—	—
22–49	3	2·3	124	13·5	59	—	13·5	—	—
28–53	5	4·0	138	20·0	48	—	11·0	—	—
53, 53	2	2·5	—	—	—	—	—	—	—
100[A]	1	4·0	203	37·9	59	—	14·5	—	—
111–192	3	9·5	196	38·5	54	26	14·1	—	—
148–178	2	4·5	190	—	36	—	—	—	—
138–236	6	11·0	179	62·5	58	27	14·0	—	—
Young at foot									
254	1	20·0	240	128·0	81	40	19·2	—	—
270	1	8·0	173	62·7	82	42	14·6	—	—
259–196	3	11·0	212	90·1	89	41	17·6	—	—
321	1	4·0	238	118·0	94	—	16·6	—	—
331[A]	1	30·0	178	92·2	58	—	14·8	4·4	2·9
			Grey kangaroo						
Pouch young									
209–236	4[B,C]	39·5	243	70·0	66	36	12·0	3·0	2·0
Young at foot									
309–328	5[C,D]	36·0	281	134·0	82	43	16·0	4·0	3·0
318	1[B]	6·0	326	190·0	94	—	25·6	8·1	4·6
498, 505	2[B,D]	43·0	253	165·0	70	45	180·0	4·0	3·0

[A] Samples taken from one animal suckling one pouch young and one young at foot.
[B] *Macropus giganteus*. [C] *M. fuliginosus*. [D] *M. giganteus* × *M. fuliginosus* hybrid.

Results

Details of the samples of milk presented for analysis and the composition and quantity of each of the major components in the solid fraction of the milk samples, are given in Table 1. Because of the limited amount of milk available in some of the samples and the number of analyses attempted there was some loss of accuracy, but where at least 30 ml milk was obtained, a more complete and accurate analysis of the major constituents and of the fat- and water-soluble vitamins was made.

In general the total solids increased in quantity with advancing age of the young. This increase was reflected in increased fat and to a lesser extent protein. However, variation in the fat content of the milk of the red kangaroo (1·4–12·8 g/100 g milk) may result, in part, from the difficulty of obtaining a representative sample of the content of the mammary gland. Nevertheless, at least in the case of fats, a species difference was apparent, with the maximum content of fat in the milk from grey kangaroos attaining 19 g/100 g milk. Differences were clearly apparent in the composition of milk, and in particular the fat content, in samples drawn from the two independent mammary glands, of one red kangaroo suckling two young aged 100 and 331 days respectively.

Table 2. Fatty acid composition of four selected samples of kangaroo milk

Additional traces (1·0%) of other long-chain fatty acids were detected in all samples; traces of lower fatty acids (12 : 0 and below) were present in two samples. Samples 1–3 from red kangaroos, sample 4 from a grey kangaroo. PY, pouch young; YAF, young at foot (age of young in days)

Fatty Acid	Trivial name	Fatty acid composition (as % of total fatty acids) in: Sample 1 PY (138–236)	Sample 2 YAF (254)	Sample 3 YAF (331)	Sample 4 YAF (318)
14 : 0	Myristic acid	2·5	1·8	1·7	1·7
16 : 0	Palmitic acid	22·5	21·5	20·5	23·0
16 : 1	Palmitoleic acid	5·1	2·6	1·6	1·5
18 : 0	Stearic acid	13·0	10·1	14·8	12·9
18 : 1	Oleic acid	42·6	50·5	52·0[A]	49·4[A]
18 : 2 18 : 3	Linoleic acid Linolenic acid	5·0[B]	8·8[B]	5·5[B]	5·5[B]
20 : 0	Arachidic acid	1·8	1·6	n.d.[C]	n.d.[C]

[A] Approximately 20% of the 18 : 1 was present in these samples as the trans isomer.
[B] Proportions of these two isomers were not resolved satisfactorily for technical reasons.
[C] n.d., not detected.

Analysis was undertaken (Table 2) of the fatty acid component of crude lipid extracted from a bulked sample from six red kangaroos all carrying a young in the latter half of pouch life and single samples of milk obtained from two red kangaroos and one grey kangaroo, all with a young-at-foot. The concentration of fatty acids was similar in all samples. Only traces of short-chain fatty acids (C_4–C_{13}) were found, while the most common saturated fatty acid (palmitic acid) averaged almost 22% of the total fatty acids followed by stearic acid at almost 13%. Of the unsaturated fatty acids, oleic acid was by far the most abundant attaining on average almost 49% of the total.

The results of analyses to determine the vitamin content of the kangaroo milk are given in Table 3. In general the vitamin content varied widely between samples so that any effects associated with the stage of lactation tended to be obscured. However, there was a trend towards an increase in the concentration of riboflavin as lactation progressed; milk from animals with 'young in pouch' and 'young at foot' contained respectively about 1·6 mg/l and 3·7 mg/l. Nicotinic acid showed the opposite trend, with a marked decline from around 50 mg/l to 10 mg/l. Pantothenic acid levels averaged 11 mg/l, there being no obvious effect due to the stage of lactation. Vitamin B_6 content of the red kangaroos' milk declined from about 1·6 mg/l 'in pouch' to 0·3 mg/l 'at foot'. Values for the grey kangaroos were respectively 1·5 and 1·0 mg/l. Vitamin B_{12} values were particularly variable with

Table 3. Concentration of vitamins in milk samples from red and grey kangaroos at different stages of lactation

— Insufficient sample available to undertake analyses

Age (days)	Riboflavin	Nicotinic acid	Pantothenic acid[A]	Vitamin B_6[B]	Vitamin B_{12}	Folic acid	Biotin	Thiamin[C]	Retinol	Carotenoids[D]	α-Tocopherol
	←(expressed as mg/l milk)							→	←(expressed as mg/kg fat)		→
Red kangaroo											
Pouch young											
11	0·4	46	5·1	0·4	0·012	0·07	0·031	0·31	—	—	—
22–49	1·3	22	—	2·0	—	—	0·060	<0·3	—	—	—
28–53	2·1	71	8·3	1·3	0·080	0·12	0·064	—	—	—	—
53	1·3	80	8·4	2·5	0·077	0·26	0·072	1·31	—	—	—
100	—	—	—	—	—	—	—	—	—	—	—
111–192	1·8	45	18·0	1·7	0·055	0·27	0·140	1·0	21	n.d.	—
148–178	1·6	32	23·0	1·6	0·006	0·32	0·055	0·92	—	—	—
138–236	1·3	35	9·4	1·7	0·014	0·17	0·083	1·5	13	n.d.	—
Young at foot											
254	4·8	10	7·2	0·26	0·055	0·13	0·052	1·5	5·4	n.d.	12·0
270	1·9	21	1·9	0·38	0·012	0·12	0·019	0·9	12·0	n.d.	—
259–296	5·2	20	11·0	0·44	0·058	0·07	0·096	2·3	7·2	n.d.	—
321	3·9	18	17·0	0·30	0·021	0·11	0·130	—	—	—	—
331	3·0	5	8·9	0·40	0·036	0·08	0·054	1·0	5·1	n.d.	—
Grey kangaroo											
Pouch young											
209–236	2·7	70	9·1	1·5	0·043	0·77	0·049	0·89	13·0	2·7	—
Young at foot											
309–328	4·0	25	10·0	1·5	0·016	0·51	0·028	0·87	8·8	1·2	—
318	4·5	3·0	19·0	1·0	0·011	0·14	0·142	2·2	15	n.d.	—
498–505	2·3	3·3	7·2	0·6	0·007	0·29	0·036	0·85	4·41	0·3	—

[A] Expressed as calcium D-pantothenate. [B] Expressed as pyridoxal hydrochloride. [C] Expressed as thiamin hydrochloride. [D] n.d., not detected.

Table 4. Comparison of the composition of milk of various species

Representative values taken from the literature and from work either published or unpublished from the National Institute for Research in Dairying, Shinfield, U.K. — Not analysed; n.d., not detected

Species:	Goat	Sow		Blue whale[A]		Black rhinoceros		Giraffe	Reindeer		Rabbit		Okapi	Rat	Red kangaroo		Grey kangaroo		Cow	Human
State of lactation (days):	Mid	Mid	Late	Unknown		300	570	150	16	91–112	7	21	Unknown	Unknown	11–234[B]	235–331[C]	209–236[B]	309–505[C]	30	30
Total solids (g/kg)	132	200	200	510	630	88	81	230	220	330	310	260	280	210	172	208	243	287	127	126
Fat (g/kg)	45	90	80	350	500	2	0	130	90	170	140	120	44	100	34·5	98	70	163	37	38
Retinol (μg/l)	574	450	320	2100	2500	n.d.	n.d.	780	675	187	2710	840	264	300	810	640	910	1590	296	182
Carotenoids (μg/l)	0	0	0	0	0	n.d.	n.d.	0	0	0	0	0	0	0	n.d.	n.d.	189	106	185	140
α-Tocopherol (μg/l)	—	—	—	—	—	n.d.	n.d.	350	—	357	—	—	860	—	—	1540	—	—	850	—
Abscorbic acid (mg/l)	17	150	130	—	—	—	17	—	—	—	—	—	—	8	—	—	—	—	20	40
Biotin (μg)/l)	48	16	20	34	65	2·9	5	9	140	140	230	140	30	85	72	70	49	69	20	5·0
Vitamin B_6 (μg/l)	67	190	220	1100	1100	200	40	500	1200	800	2900	2500	5300	790	1600	360	1500	1030	600	110
Vitamin B_{12} (μg/l)	0·4	1·2	1·4	9	8	1·1	5	11	14	12	90	50	90	6–83	41	36	43	11	3	0·23
Folic acid (μg/l)	9·5	—	5	—	—	—	—	—	—	—	100	—	—	180	200	100	770	310	50	50
Nicotinic acid (mg/l)	1·9	9·4	12	14	27	0·10	0·09	2·1	1·29	1·56	8·4	8·7	0·8	18·1	47	15	70	10	0·80	1·7
Pantothenic acid (mg/l)	2·9	4·6	5·3	8·5	18	3·4	3·4	2·2	4·9	7·2	15·8	7·3	21	5·7	12	9·2	9·1	12·1	3·5	2·6
Thiamin (mg/l)	0·41	—	0·7	1·2	1·6	0·25	0·90	0·70	1·4	1·4	1·7	1·7	1·2	1·5	0·89	1·43	0·89	1·31	0·44	0·17
Riboflavin (mg/l)	1·84	—	1·8	1·2	1·3	0·30	0·60	1·53	5·6	10·0	2·3	2·8	10	1·1	1·4	3·8	2·7	3·6	1·7	0·34

[A] Two samples. [B] Pouch young. [C] Young at foot.

no obvious lactational trend among the red kangaroos (overall mean value 0·039 mg/l) whereas the mean value from the grey kangaroos with 'young at foot' (0·011 mg/l) was much lower than for the red kangaroos (0·036 mg/l). Folic acid values were also very variable, with an overall mean of 0·23 mg/l. However, there was an indication that values from animals with 'young at foot' tended to be lower than those with 'young in pouch'. The milk from kangaroos suckling young in the pouch was much richer in folic acid for grey kangaroos (average 0·77 mg/l) than for the red kangaroos (average 0·2 mg/l). Biotin values averaged overall 0·069 mg/l. Thiamine values in early lactation were low, around 0·3 mg/l, but increased to around an average 1·27 mg/l and ranged from 0·85–2·3 mg/l. Table 3 shows that the retinol concentration in the fat of milk from both red and grey kangaroos decreased as lactation progressed but because of the increase in fat concentration throughout lactation no similar trend is seen in Table 4 where the concentration is expressed as μg/l milk. The α-tocopherol concentration of the only sample with sufficient material for this analysis was 12 mg/kg fat or 1540 μg/l milk. Carotenoids were only detectable in the milk where sufficient material was available for analysis.

Discussion

Since the collection of milk samples analysed in this study, more recent investigations (Griffith *et al.* 1972; Messer and Green 1979; Green *et al.* 1980) have revealed aspects of qualitative changes in milk drawn in sequential samples from kangaroos throughout lactation. As the aim of this study was to collect over the shortest possible time sufficient milk at known stages of lactation, to permit as many assays as possible, including for the first time, assessment of the vitamin content, it was not possible to monitor in such detail changes in the composition of the milk throughout lactation. Despite their uneven distribution throughout lactation and a bias towards values obtained with a greater volume of milk available in late lactation, the results of the analysis confirmed those obtained by more frequent sampling in that the concentration of milk solids gradually increased with advancing age of the suckled young. These changes reflected an increasing content of fat and to a lesser extent casein and total protein. Insufficient milk was available from the teat suckled by the younger animals to compare the relative concentrations of palmitic and oleic acid in early and late milks as demonstrated by Griffiths *et al.* (1972). Certainly all four samples analysed (Table 2) exhibited a greater quantity of oleic acid than palmitic acid, as expected from glands in the latter period of lactation.

For comparison, representative values for the composition of milk of various species are given in Table 4 along with mean values obtained from the individual values given in Tables 1 and 3 for the milk of the kangaroos.

The total solids and fat content of kangaroo milk increase as lactation progresses. It is richer in these components than cow or human milk but well in the range seen for the other species shown except the black rhinoceros, where the total solids are low due to the complete absence of fat.

Kangaroo milk is richer in retinol than most other species listed in Table 4 except the blue whale and the rabbit. The milk of grey kangaroos contains as much carotenoids as cows' milk. Unfortunately, in only one instance was enough material available for a determination of the α-tocopherol content. The concentration found in the fat from this sample of kangaroo's milk, 12 mg/kg, was lower than in the fat of cows' milk but owing to the high fat content of the sample the kangaroo's milk contained nearly twice as much tocopherol as cows' milk.

Biotin and vitamin B_6 concentrations vary considerably among the listed species. For instance, vitamin B_6 values range from 67 μg/l milk in the goat to 2900 μg/l in rabbits' milk. The milk of the kangaroos contains biotin and vitamin B_6 in concentrations within the range found in other animals. Again, vitamin B_{12} shows a wide spread of values but the concentration of this factor in the milk of the kangaroos more closely resembles that of the rat, rabbit and reindeer than other species. Except for the rabbit, kangaroo milk is richer in folic acid than the other species for which values are provided. Only the milk of the blue whale and the rat approach the high concentrations of nicotinic acid found in both red and grey kangaroos. The milk of the okapi contains even more pantothenic acid than the kangaroos, but the riboflavin and thiamin concentrations fall within the range found in other species.

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