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Southern elephant seal, *Mirounga leonina*: composition of milk during lactation

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Abstract An analysis of milk constituents during various stages of lactation in the southern elephant seal *Mirounga leonina* was carried out. Forty-six milk samples were taken from 30 females throughout lactation during 1985, 1987, 1990 and 1991 on Stranger Point, King George Island, South Shetland Islands. Total nitrogen (TN), non-protein nitrogen (NPN), sugar, fat, ash and water were measured, and from some of these data true protein and energy content were calculated. The results showed a high degree of variation in water and fat concentrations among samples at different stages of lactation. During the first 20 days the fat content of milk increased from about 12 to approximately 52%, while water content fell from 70 to 33%. The composition of milk changes rapidly during the first days post- partum. Protein, minerals and sugar appear to remain stable after the fourth day of lactation. Milk samples contain significant levels of sugars; thin layer chromatography indicates the presence of lactose and glucose together with other unidentified components. There is evidence of a striking change in composition of the milk in the later part of lactation; the progressive increase in the fat:water ratio is abruptly reversed just prior to weaning.

Introduction

Lactation is widely variable among mammals. Most mammals lactate for relatively long periods, do not fast during lactation, and wean their pups gradually. In contrast many Phocidae have a short suckling period during which they fast, while producing energetically rich milk (Bonner 1984). Pups have a high rate of growth at this time. Southern elephant seal (*Mirounga leonina*) pups are solely dependent upon milk from their mothers for nutrition and growth during the lactation period. The females fast until their pups are weaned (Oftedal et al. 1987). Pups are born weighing approximately 50 kg and they increase by 80–200 kg until weaning, i.e. during the 21–24 days of the nursing period (Oftedal et al. 1987).

Bryden (1968) performed the first study on the composition of southern elephant seal *M. leonina* milk at Macquarie Island. Since then, to our knowledge, only one study has been carried out (Peaker and Goode (1978), erroneously titled as a study on fur seals; the correction appears in Bonner (1984) in Table II and in his literature citation. The purpose of our paper is to report the results of analysis carried out to determine the main nutrients in southern elephant seal milk during the course of lactation, by sampling females of known parturition date in their natural habitat, on Stranger Point, King George Island.

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Materials and methods

Milk samples were collected at Stranger Point, King George Island, South Shetland during the 1985, 1987, 1990 and 1991 breeding seasons. To minimize variations among samples, milk collection was standardized.

Collection time was fixed at about 3 to 6 p.m.. A total of 46 samples from 30 mothers of female pups of known birth date were collected. Milk samples were taken at 4 different stages of lactation from 1 female, at 3 stages from 3 females, and 2 stages from 7 females. In addition, single samples were obtained from 19 females at different stages of lactation. The females were immobilized with Rompun (xilazina hydrochloride) using an intramuscular dose of 3–3.9 mg/kg during 1985 and 1987, and ketamine hydrochloride using an intramuscular dose of 2.42–8.48 mg/kg body weight during 1990 and 1991. No method was available for direct weighing of the females but body weight was estimated by substitution for standard length in regression equations (Vergani and Spairani 1980). Females were milked with a manual vacuum pump 10 min after an intramuscular injection of 60 units of synthetic oxytocin (Bryden 1968). Pup weights and durations of suckling periods were recorded.

Milk from both glands was mixed and the entire sample was frozen in tightly capped containers at −20°C for periods ranging from 3–6 months. Average sample volume was approximately 80 ml (range 40–160 ml).

Biochemical analysis

Samples of whole milk were thawed and warmed to room temperature (approximately 25°C) and homogenized. The following methods were used for analysis:

Water: drying to constant weight in an oven at 100°C.
Ash: incineration of dry solids overnight at 500°C in a muffle furnace.
Fat: analysed gravimetrically by an ether extraction method (Emery et al. 1978)
Reducing sugars: determined by the chloramine – T method (Norma Internationale FIL-IDF 1974), the reducing power is arbitrarily expressed as lactose. The carbohydrates present in deproteinized samples were also examined by thin layer chromatography on silica gel sheets developed with 1-butanol-pyridine – water (6:4:3) and visualized with naphthoresorcinol-sulphuric acid, aniline-diphenylamine – phosphoric acid, and the ninhydrin reagent to distinguish total sugars, reducing sugars and amino sugars respectively. Individual sugars were identified on the basis of the Rf values by parallel runs with reference substances.
Total nitrogen (TN): microkjeldahl (Association of Official Analytical Chemists 1980). Non-protein nitrogen (NPN): determined after precipitating milk proteins with 24% trichloroacetic acid. The supernatant was digested by the Kjeldahl procedure (Lonnerdal et al. 1976).
Total protein: calculated as 6.38 × TN.
True protein: calculated as 6.38 × (TN − NPN).
All assays were performed in duplicate or triplicate and mean values for each sample utilized in final data analysis. Caloric values of 9.3 kcal/g for fat, 4.1 kcal/g for carbohydrate and 5.4 kcal/g for protein (FAO/OMS/UNU 1985) were used for calculation of the energy content of milk.

Results

Data on energy content together with gross composition of whole milk during lactation are reported in Table 1 and Fig. 1. Milk composition changed rapidly during the first 3–4 days post-partum. The concentration of water decreased after the second day, while the fat concentration increased. TN and NPN concentrations were higher in the first few days post-partum, decreasing thereafter (Table 2, Fig. 2). Minerals were also in higher concentrations during this short period (Table 1). These components appeared to remain stable after the fourth day of lactation.

The results revealed that water concentration had an inverse relationship to the amount of fat present in the milk. Water declined from birth until

Table 1 Concentration of milk components (mean ± SD) throughout the lactation period

Age of Pup (Days)	Number Samples	Water[a]		Fat[a]		Reducing[a] Sugars		Ash[a]		Total[a] Protein		Gross Energy Kcal (mean)
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
1	1	69	–	12.98	–	3.82	–	1.04	–	12.97	–	206.4
2	5	70.59	6.28	12.11	4.85	3.48	0.43	1.01	0.1	11.7	0.68	190.1
4	2	50.38	6.99	32.06	3.46	1.96	0.08	0.82	0.01	10.05	0.28	360.5
5	1	43.31	–	37.36	–	2.51	–	0.82	–	10.4	–	413.9
6	1	45.58	–	39.4	–	1.69	–	0.76	–	9.95	–	427.1
7	6	45.78	6.34	39.13	6.51	2.55	0.33	0.64	0.06	10.21	0.88	429.5
10	1	36.33	–	46.47	–	1.86	–	0.7	–	9.58	–	491.5
11	1	42.19	–	39.4	–	3.26	–	0.73	–	9.77	–	432.5
12	1	36.43	–	45.21	–	3.27	–	0.74	–	11.36	–	495.2
14	10	37.38	5.55	48.75	6.27	2.74	0.81	0.7	0.13	9.15	1.12	514.0
16	1	39.12	–	44.98	–	2.7	–	0.69	–	11.45	–	491.2
17	5	33.44	4.02	51.69	4.47	1.99	0.71	0.64	0.06	8.45	0.73	534.5
18	1	35.7	–	49.85	–	2.01	–	0.68	–	10.16	–	526.7
19	1	36.39	–	49.86	–	2.82	–	0.68	–	9.97	–	529.1
20	5	38.86	5.37	44.51	7.9	2.66	0.67	0.68	0.09	10.72	2.11	482.7
23	4	46.9	7.31	35.51	6.95	2.9	0.6	0.62	0.06	10.91	0.99	382.4

[a] Percentage by wet weight in whole milk

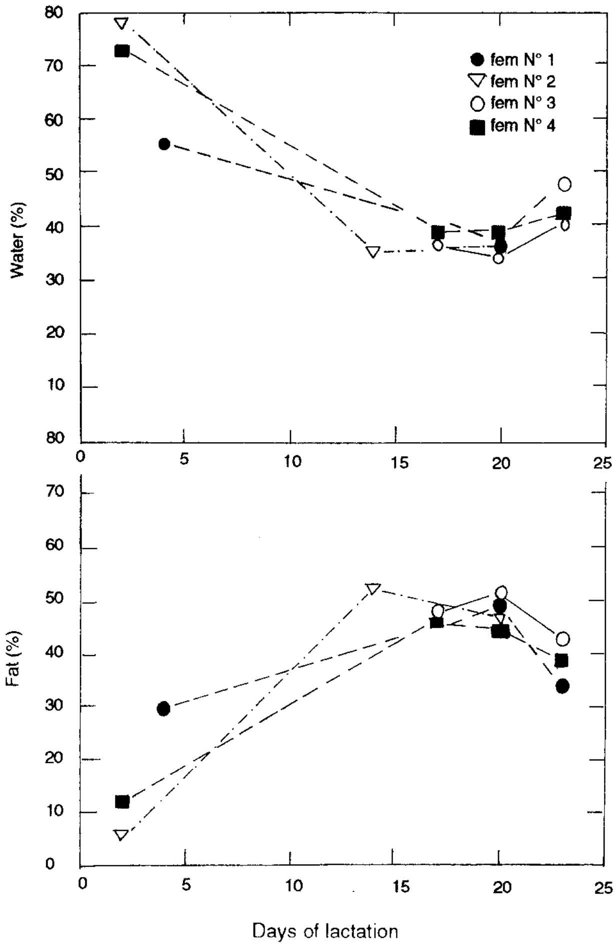

Fig. 1 Southern elephant seal females sampled sequentially throughout lactation. The values refer to percentage by wet weight in whole milk

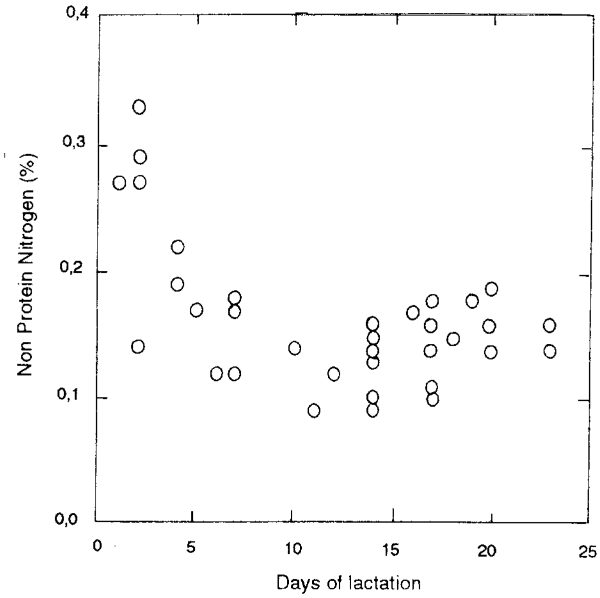

Fig. 2 Changes in non-protein nitrogen content of milk of southern elephant seals as lactation progresses. The values refer to percentage by wet weight in whole milk

approximately 17–20 days post-partum, while during the same period fat content rose. Carbohydrate content varied between 1.69–3.82% of the milk by wet weight. Although no trend was observed in this component during lactation (Table 1), higher values were found in the first two days of the nursing period. Thin layer chromatography of samples revealed at least eight sugar components, six of which were reducing sugars, including lactose and glucose. Four components were characterized as amino sugars, two being reducing sugars. The procedure would exclude any oligosaccharides present since they are not very mobile in the usual sugar solvents.

Table 2 shows that nitrogen components exhibited a slight variation among samples from different

Table 2. Nitrogen and protein contents of milk during lactation

Age of Pup (Days)	Number Samples	Total[a] Nitrogen Mean	SD	Non Protein[a] Nitrogen Mean	SD	Protein[a] Nitrogen Mean	SD	True[a] Protein Mean	SD
1	1	2.03	–	0.27	–	1.76	–	11.23	–
2	5	1.83	0.1	0.26	0.07	1.57	0.14	10.04	0.89
4	2	1.58	0.05	0.20	0.02	1.37	0.07	8.74	0.45
5	1	1.63	–	0.17	–	1.46	–	9.31	–
6	1	1.56	–	0.12	–	1.44	–	9.19	–
7	6	1.6	0.14	0.17	0.02	1.43	0.13	9.1	0.86
10	1	1.5	–	0.14	–	1.36	–	8.68	–
11	1	1.53	–	0.09	–	1.44	–	9.19	–
12	1	1.78	–	0.12	–	1.66	–	10.59	–
14	10	1.43	0.17	0.13	0.03	1.3	0.17	8.3	1.1
16	1	1.8	–	0.17	–	1.63	–	10.4	–
17	5	1.48	0.1	0.14	0.03	1.34	0.12	8.56	0.76
18	1	1.59	–	0.15	–	1.44	–	9.19	–
19	1	1.56	–	0.18	–	1.38	–	8.8	–
20	5	1.68	0.33	0.15	0.02	1.53	0.34	9.75	2.14
23	4	1.71	0.16	0.14	0.01	1.57	0.17	10.0	1.06

[a] Percentage by wet weight in whole milk

mothers on the same day of lactation, and among samples of different stages of lactation in the same mother. The NPN concentration range was 5.88 to 14.21% of the TN.

Data also revealed a striking difference in composition of the milk in the latter part of lactation, from the 20th to the 23rd day of suckling: the progressive increase in the fat: water ratio is abruptly reversed just prior to weaning (Table 1, Fig. 1). This trend was corroborated by repeated samples on four females (Fig. 1).

Discussion

An inverse relationship between water and fat concentration has been noted in phocidae seal milk (Bryden 1968; Kooyman and Drabek 1968; Le Boeuf and Ortiz 1977; Riedman and Ortiz 1979). Examination of the results in Table 1 showed that there was a variation between samples from different mothers on the same day of lactation (Vergani et al. 1989). Milk composition changes markedly through the course of lactation in several pinniped species. Northern elephant seal shows an important increase in fat content from birth to 2 weeks post-partum, but remaining relatively constant during the last part of the lactation period (Riedman and Ortiz 1979). There appears to be a general trend towards an increase in milk solids during lactation in these species, which tends to be accentuated in those species which apparently fast during nursing. This is commonly interpreted as a way of conserving water under water restriction regimes (Riedman and Ortiz 1979). A similar but less pronounced rise in fat content in the first few weeks post-partum is found in both Weddell seal (Tedman 1985) and harp seal (Oftedal et al. 1987). The hooded seal exhibits little change in milk composition during its 4-day lactation period, and no trends are apparent in fat content (Oftedal et al. 1987).

Our results confirmed those observed by Bryden (1968) and Peaker and Goode (1978); although the samples described in this last paper were attributed to the fur seal, in fact they were from the southern elephant seal (Bonner 1984). Changes in the fat content of southern elephant seal milk during lactation paralleled those observed in other seals, except just prior to weaning. Fat levels rise from birth to the second week, before falling again at weaning. The decline in fat content beyond 2 weeks post-partum observed by Bryden (1968) was confirmed by our study, showing a similar trend but with smaller decreasing values in the fat/water ratio (Fig. 1, Table 1). Peaker and Goode (1978) did not remark on this fall in fat content at the end of lactation, although they also found the fat levels on day 26 of lactation lower than those on day 17.

The increasing fat and decreasing water content of seal milk during early lactation may be important in maintaining the water balance of the fasting lactating female. However, the water conservation hypothesis seems to be of limited application since it does not provide a satisfactory explanation for results found in southern elephant seal milk near weaning. The net amount of energy utilized for maternal maintenance increases with increasing lactation duration as the energy available for milk production steadily decreases (Costa 1993). The only water available for milk production in the fasting lactating female is the water produced while fat is metabolized via oxidation, minus the amount minimally required for its own physiological maintenance (Riedman and Ortiz 1979). Quantitative differences in the composition of milk may arise from differences in the rate of biosynthesis and secretion of the components, including water (Jenness 1986). A decreased rate of fat synthesis and secretion could produce a decreasing fat content in milk, but no conclusive evidence exists on this point.

Data from other species indicate that significant compositional changes occur during either the rapid or gradual involution of the mammary gland that accompanies weaning. These changes are due to a progressive decline in the gland's synthetic activity, an increase in paracellular transport processes over the normally predominant transcellular mechanisms, and changes in water transport (Lascelles and Lee 1978). These responses result in glandular characteristics that are, in some respects, similar to those observed in early lactation (Peaker 1978). The relationship of these changes to the involution of the mammary gland require further study, necessary to the understanding of the weaning process. On the other hand, the lactating female comes into oestrus shortly before weaning, is mated several times, and after weaning leaves for the sea (Bonner 1984). The role of prolactin and other hormones in the control of lactation, the maintenance of milk secretion by the mammary gland and the mammary involution in pinnipeds is not known (Oftedal et al. 1987). Further physiological studies are required before we can adequately explain the variability of changes in composition of milk observed in southern elephant seals. No change was found in the other nutrients during this latter part of lactation. High fat and protein levels, along with low water and sugar levels, are characteristic of the milk of marine mammals (Jenness et al. 1981). The chief function of fat in milk is to supply energy; protein is a source of amino acids for incorporation into body protein of the growing nursling. Pups of the studied species require a high-energy milk to attain rapid growth, which involves deposition of fat to a large extent. The high total solids content may be related to the conservation of water (as discussed earlier) and a demand to produce a more concentrated and high-energy milk. *M. leonina* pups become completely independent after weaning and require an energy store prior to feeding at sea, because they fast for at least 25 days post-weaning.

Protein values resembled those observed in the milk of other marine mammals, and are higher than the values reported for most terrestrial species (Ling et al. 1961; Jenness and Sloan 1970; Hibberd et al. 1982). This may be related to the rapid growth and development of the pup which is at least partially dependent on an adequate supply of nitrogen. A considerable fraction of the total nitrogen in milk is present as NPN (Table 2). The NPN fraction consists of diverse groups of compounds, which have only been partially characterized in other species: urea, uric acid, ammonia, creatinine, free amino acids, peptides, as well as polyamines, hormones, growth factors and N -containing oligosaccharides (Donovan and Lonnerdal 1989). Protein nitrogen (PN) reflects the true protein content of milk. The amount of PN remained fairly constant throughout lactation.

Lactose is the main carbohydrate present in milks of mammals, except in certain pinnipeds. The first demonstration of absence of lactose from the milk of any mammal was in the California sea lion (Pilson and Kelly 1962). However, the methods available at that time were such that small amounts of lactose might have been missed. The composition of milk from some aquatic species indicates the presence of significant levels of either reducing or neutral sugars, and the presence of lactose (Jenness and Sloan 1970; Jenness and Odell 1978). Peaker and Goode (1978) and Riedman and Ortiz (1979) reported no lactose or only traces of sugars present in the southern and northern elephant seals milk, respectively. Our analysis of southern elephant seal milk showed low but significant amounts of carbohydrate, similar to those of grey seals and Weddell seals (Jenness and Sloan 1970).

Chromatographic analysis of sugars showed a number of components present in milk; a considerable amount of sugars remained at the origin without resolution, probably indicating that they were oligosaccharides. Although lactose is not the predominant sugar, the carbohydrate values were reported here as "lactose" since most of the individual sugars have not been identified yet. The result is likely to err since most of the non-lactose carbohydrates have different reducing power from lactose or are not included in values obtained by reduction methods. Further research is in progress in order to identify the nature of carbohydrates and to more accurately quantitate their concentration in milk.

The estimated caloric content of milk is much higher than that of terrestrial mammals previously investigated (Jenness and Sloan 1970; Jenness et al. 1981; Kretzmann et al. 1991; Puppione et al. 1992). For species of arctic, antarctic or aquatic habitats, milk calories are principally derived from fat (Blaxter 1961; Jenness 1986). In *M. leonina* this reflects an intense and efficient nursing regime that permits the female, who fasts during lactation, to transfer a large amount of energy to her pup in a short period. In our samples, fat is providing 59 to 90% of the total calories, a range of 8 to 34% from proteins and 1 to 7% from sugars, according to the stage of lactation. Most of the nutrient concentrations reported here are in accordance with the general pattern of pinniped milk composition.

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