RESEARCH ARTICLE

Milk Composition in Free-Ranging Polar Bears (Ursus maritimus) as a Model for Captive Rearing Milk Formula

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The goals of this study were to have an improved understanding of milk composition and to help create a suitable milk formula for cubs raised in captivity. Milk samples were evaluated for fat, fatty acids, carbohydrate, vitamin D3, 25(OH)D3, vitamin A (retinol), vitamin E (α-tocopherol), protein, and amino acids. Total lipids in milk did not differ for cubs (mean ± SEM = 26.60 ± 1.88 g/100 ml vs. yearlings 27.80 ± 2.20 g/100 ml). Milk lipids were of 23.6% saturated fatty acid for cubs and 22.4% for yearlings. Milk consumed by cubs and yearlings contained

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43.8 and 42.0% mono-unsaturated fatty acids and 23.4 and 21.9% polyunsaturated fatty acids, respectively. Carbohydrate content was higher in milk for cubs (4.60 ± 0.64 g/100 ml) than for yearlings (2.60 ± 0.40 g/100 ml). Vitamin D₃ concentration of milk was 18.40 ± 5.00 ng/ml in early lactation compared with 7.60 ± 2.00 ng/ml for mid-lactation. 25(OH)D₃ was lower in milk consumed by cubs (162.00 ± 6.70 pg/ml) than in milk consumed by yearlings (205.00 ± 45.70 pg/ml). Vitamin A concentrations were 0.06 ± 0.01 and 0.03 ± 0.01 μg/ml for cubs and yearlings, respectively. Vitamin E was higher in milk consumed by cubs (20.16 ± 4.46 μg/ml) than by yearlings (7.30 ± 1.50 μg/ml). Protein content did not differ in milk available to cubs (11.40 ± 0.80 g/100 ml compared with milk for yearlings 11.80 ± 0.40 g/100 ml). Taurine was the most abundant free amino acid at 3,165.90 ± 192.90 nmol/ml (0.04% as fed basis). Zoo Biol 29:1–16, 2011. © 2011 Wiley-Liss, Inc.

Keywords: amino acids; bears; fatty acids; hand-rearing; lactation; maternal milk; vitamin A; vitamin D; vitamin E

INTRODUCTION

Pregnant female polar bears (Ursus maritimus) require large amounts of stored fat after breeding in the spring to successfully overwinter in dens and rear cubs without access to food [Watts and Hansen, 1987]. Hunting on the sea ice in spring provides access to the primary prey of polar bears, ringed seals (Pusa hispida), and bearded seals (Erignathus barbatus) [Smith, 1980; Derocher et al., 2002; Thiemann et al., 2008]. Polar bears from Svalbard also hunt and consume harp seals (Phoca groenlandica) and reindeer (Rangifer tarandus platyrhynchus) as well as birds and their eggs [Stempniewicz, 1993; Derocher et al., 2000, 2002]. Unlike brown bears (Ursus arctos), only pregnant female polar bears enter dens to produce cubs, whereas all other polar bears remain active during winter [Watts and Hansen, 1987; Ramsay and Stirling, 1988]. In autumn, pregnant female polar bears enter winter dormancy, live off fat stores, have a specialized fasting physiology, and slower heart rate [Ramsay and Stirling, 1988; Ramsay et al., 1991; Amstrup, 2003]. Mothers nurse their young in dens and continue to nurse their young after leaving the dens for up to 2½ years [Ramsay and Stirling, 1988; Derocher et al., 1993a].

Polar bear milk is similar in composition to the milk of aquatic marine mammals [Jenness et al., 1972; Craighead, 2003], i.e. high in fat and protein content and low in carbohydrates [Jenness et al., 1972; Urashima et al., 2003]. Previous data for milk composition of polar bears in studies by Baker et al. [1963], Cook et al. [1970], and Jenness et al. [1972] utilized 10 samples collected during lactation (7–29 months). New data emerged evaluating early and mid-lactation samples as described by Derocher et al. [1993a] and Kenny et al. [1998] with a much larger sample pool >100 and all five references were used to design hand-rearing formulas for polar bear cubs in captivity [Hedberg, 2002; Slifka and Jacobson, 2005].

Often lipid sources, including whole cream or half-and-half (a commercial 1:1 mix of whole milk and cream), were used to increase the fat concentration of milk formulas for hand-reared polar bear cubs. Unfortunately, when trying to duplicate milk fat composition, reported to average 30% [Baker et al., 1963; Cook et al., 1970; Jenness et al., 1972; Derocher et al., 1993a], the recipe had poor acceptance and cubs fed such a formula required medical intervention [Hedberg et al., 2006]. There is
limited information on the fatty acid (FA) profile of polar bear milk and the published results may have analytical errors [Iverson and Oftedal, 1995]. Alternative ingredients such as fish oil may prove more suitable because the FA profile of fish oil more closely reflects natural diets and the resulting lipid stores in milk and adipose tissues of free-ranging polar bears [Pond et al., 1992; Hedberg et al., 2006].

Five of the last 20 hand-reared polar bear cubs less than 3 months of age developed metabolic bone disease that was linked to nutrient deficiencies [Hedberg et al., 2009]. In 1994, these deficiencies were corrected in two hand-reared cubs at the Denver Zoo, Denver, CO by supplementing the milk formula with cod liver oil [Kenny et al., 1999] as a source for increasing fat [Hedberg, 2002] and especially the long-chain omega-3 FA and vitamin D [Kenny et al., 1998].

The study of Derocher et al. [1993a] provides reasonably complete information on the composition of polar bear milk for total solids, total protein, total lipids, ash, carbohydrates, and gross energy. However, limited data are available for Vitamin D₃ [Kenny et al., 1998] and there is also a lack of information on the other fat-soluble vitamins including vitamin A (retinol) and vitamin E (α-tocopherol). Similarly, there are no published data on the complete fatty acid and free amino acid composition of polar bear milk.

The amino acid taurine is sometimes in short supply in human infant formulas [Chesney et al., 1998], and human infants fed a taurine-free formula were reported to have vitamin D deficiency with nutritional rickets [Zamboni et al., 1993]. Bile salts conjugated with taurine or glycine are important in the absorption of fat and fat-soluble vitamins [Thompson, 1971; Pellicoro and Faber, 2007]. A commercial canine milk formula often used for hand-rearing carnivores, often combined with cows’ milk for ursids, contained only minimal amounts of taurine [Spitze et al., 2003; Hedberg et al., 2007]. The low taurine concentrations may have implications for adequate absorption and utilization of fat-soluble vitamins even with no other apparent taurine deficiency [Hedberg et al., 2007]. Taurine is a common supplement in artificial marine mammal milk formulations because it is the most abundant free amino acid in cetacean milk [Walsh and Rogers, 1995], but there are no published data on the taurine content of polar bear milk. However, in bears, bile acids are conjugated solely with taurine [Hagey et al., 1993].

Climate change is an increasing concern for the conservation of polar bears [Stirling and Derocher, 1993; Derocher et al., 2004; Durner et al., 2009]; hence, the need for captive rearing of polar bears may become a part of conservation and management plans. Successful rearing of polar bears requires improved milk formula to meet the nutritional requirements of cubs during their growth period to improve their survival and support normal growth and development. Milk formulas must contain the proper nutrient and energy content to meet the requirements of growth. To reach this goal, improved data on the composition of milk from early and mid-lactation of polar bears are needed. The objective of this study was to obtain information on the composition of fat, fatty acids, carbohydrate, fat-soluble vitamins, protein, and amino acids because all these constituents are required nutrients.

**METHODS**

Milk samples were collected in April 2000–2002, in Svalbard, Norway (74–79°N, 15–25°E) as part of a long-term monitoring program of polar bears. Free-ranging polar bears were caught on sea ice by remote injection of a dart (Palmer Cap-Chur Equipment,
Douglasville, GA) containing the drug Zoletil® (Virbac, Carros, France) fired from a helicopter [Stirling et al., 1989]. Animal handling methods were approved by the National Animal Research Authority (Norwegian Animal Health Authority, P.O. Box 8147 Dep., N-0033 Oslo, Norway). Age was determined from a rudimentary premolar tooth extracted from bears >1 year old and cubs and yearlings were aged by tooth eruption patterns. The sex, reproductive status, and a series of standardized morphometric measures were collected for each bear. Milk samples were collected from females with one or two cubs, after an intramuscular injection of 2.0 ml oxytocin (20 IU/ml). There was a 30–70 min range from time of capture to milk sample collection that allowed for maximum drug effect and mammary gland refill time. Milk was expressed manually from all four mammae into clean sampling vials and then stored frozen at −20 and −70°C until analysis. Efforts were made to collect milk from all mammary glands but because of variation in the suckling and use patterns of offspring before capture, it was not always possible. The samples contain the natural range of variation in composition from free-ranging bears and do not allow for controlled study conditions. This is an issue for all wild collection studies and is a potential source of error. The milk samples represent what cubs would be exposed to during nursing cycles. The volumes of milk collected from the polar bears were generally small and varied from 5 to 30 ml.

Each of the sixteen 5 ml samples was thawed and 1-ml aliquots were prepared using a positive-displacement pipetting system. Samples from females with cubs were classed as early lactation and females with yearlings as mid-lactation. Milk analysis included fat and fatty acids, carbohydrate, vitamin D³ and 25(OH)D³, vitamins A and α-tocopherol, and protein and amino acids.

Fat and Fatty Acid Analysis

FA composition of polar milk was determined by using gas chromatography of methyl esters as described by Sukhija and Palmquist [1988] with minor modifications [Crocker et al., 1998; DePeters et al., 2001].

Carbohydrate Analysis

Total carbohydrates in polar bear milk samples were analyzed by the orcinol–sulphuric acid method as described by Svennerholm [1956] with minor modifications [Polberger and Lönnnerdal, 1993].

Vitamin D₃, 25(OH)D₃ Analysis

Milk levels of vitamin D₃ and 25(OH)D₃ were measured using previously described methods that include methanol:methylene chloride extraction, precipitation with cold methanol and ether, and an alkaline buffer wash [Hollis, 1983, 2005]. The samples were then passed through a silica chromatography cartridge and the eluted vitamins were subjected to normal phase and reverse phase high-performance liquid chromatography. Final quantitation was carried out by competitive protein binding.

Vitamin A and Vitamin E Analysis

Samples were thawed and 100 µl milk aliquots were mixed with 100 µl methanol and 50 µl δ-tocopherol acetate (internal standard). Samples were extracted three times with 0.25 ml hexane. The hexane layer was removed, combined, and
evaporated under a gentle stream of nitrogen. The residue was dissolved in 100\,\mu l 2-propanol.

Vitamin A (retinol) and vitamin E (\alpha-tocopherol) were determined by reversed-phase isocratic high-performance liquid chromatography (HPLC). Vitamins were separated on a Waters C18 Resolve Column (15 cm \times 3.9 mm; Millipore, Milford, MA) protected with an Upchurch C18 guard column (Upchurch Scientific, Oak Harbor, WA). A mobile phase of methanol/water (98/2) at 1 ml/min was used to elute the vitamins. Retinol was measured by absorbance at 325 nm and \alpha-tocopherol by absorbance at 290 nm. When possible, each analytical run was repeated four times and the averages were calculated.

**Protein and Amino Acid Analysis**

Milk samples were evaluated for total protein using the modified Lowry method [Peterson, 1977] as modified by Polberger and Lönnerdal [1993]. Equal volumes of milk and 6\% sulfosalicylic acid were added to the thawed milk samples. After mixing, samples were centrifuged and the supernatant was stored at −20°C for amino acid analysis. The quantity of each amino acid was determined colorimetrically using ninhydrin for color development (Amino acid analyzers, Model 6300 Amino Acid Analyzer; Beckman Instruments, Palo Alto, CA or Biochrom Ltd., Cambridge, UK).

**RESULTS**

Milk samples were obtained from 16 females (11 with cubs and 5 with yearlings) captured between late March and early May. Ages of cubs were estimated based on expected birth dates for cubs in the population which is approximately January 1 and capture month. Milk samples from females with cubs <4 months of age were defined as early lactation and those from females with yearlings >16 months of age were defined as mid-lactation.

Milk composition results for total fat, fatty acids, carbohydrate, fat-soluble vitamins, protein, and the sulfur amino acid taurine were similar for milk from bears with either cubs or yearlings (Table 1). A comparison of current results with historical data (Table 2) shows the lack of reporting for fat-soluble vitamins A, D, and E and taurine. Figure 3 presents the comprehensive milk FA signature of 14 bears consuming a marine diet as well as a distinct difference in FA profile from two bears that were suspected to be consuming an ungulate prey species, a proposal which was supported by the fact that these two bears were caught in proximity to reindeer carcasses.

**Fat and FAs**

Mean total fat content was not significantly different (\textit{t}-test, \textit{t} = 0.37, df = 14, \textit{P} = 0.72) and was 26.7\% for mothers with cubs and 27.8\% for mothers with yearlings. The range was 12.2–37.9\% with an overall mean of 27.1\%.

The fatty acid composition for saturated fatty acids (SFA) showed palmitic acid (C16:0) to be in highest concentration. Milk fat ranged from 8.4 to 40.5\% for C16:0 with an overall mean of 14.9±2.0\%. Oleic acid (C18:1), a mono-unsaturated fatty acid (MUFA), ranged from 14.9 to 33.9\% with a mean of 21.6±1.08\%. Palmitoleic (C16:1), another (MUFA), ranged from 3.8 to 16.2\% with a mean of...
Linolenic acid (C18:3n3), an omega-3 (n3), polyunsaturated fatty acid (PUFA) ranged from 1.23 to 11.3% with a mean of 6.66%.

Docosahexaenoic acid (C22:6), a long-chain omega-3 (PUFA), ranged from 0.59 to 10.6% with a mean of 7.55%.

The overall comparative distribution of SFA, (MUFA), and (PUFA) between cubs and yearlings was similar (Fig. 1).

**Carbohydrate**

The carbohydrate content of milk samples produced during early lactation was almost two-fold higher than that of milk from mid-lactation although not quite significantly different ($t$-test, $t = 2.00$, df = 14, $P = 0.065$). Total carbohydrate concentrations were considerably higher than in most published studies. This is possibly due to methodological differences because there was a large variation among earlier studies.

**Vitamins**

Table 1 shows higher concentrations of vitamins A, D$_3$, and E found in early lactation milk samples compared with mid-lactation samples. 25(OH)D$_3$ levels in milk from both stages of lactation did not differ significantly ($t$-test, $t = 1.32$, df = 12, $P = 0.21$). Similarly, there were no significant differences between lactation stages in vitamin A concentrations ($t$-test, $t = 2.03$, df = 12, $P = 0.065$) or for vitamin E concentrations ($t$-test, $t = 1.77$, df = 12, $P = 0.10$).

The total vitamin D$_3$ content of polar bear milk was measured in 14 milk samples. The concentrations of the prohormone vitamin D$_3$ and 25(OH)D$_3$ are expressed in ng/ml and pg/ml, respectively, as well as IU/l (Table 2). The range of values was great (149–2134 IU/l), and they were much higher than vitamin D$_3$ concentrations found in human or unsupplemented cows’ milk [Reeve et al., 1982a,b; Hollis and Wagner, 2004]. The total vitamin D$_3$ content of milk from females nursing cubs was higher than milk provided to yearlings, but the differences were not statistically significant.

Vitamin D$_3$ is soluble in lipids, but there was no correlation between vitamin D$_3$ and percent fat in the samples.
<table>
<thead>
<tr>
<th>Stage of lactation (months)</th>
<th>Averages from this survey</th>
<th>Averages from multiple studies</th>
<th>Averages from selective data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n = 16$</td>
<td>$n = 120^{a,b,c,d,e}$</td>
<td>$n = 10^a$ $n = 40^b$ $N = 60^b$ $n = 7^c$ $n = 1^d$ $n = 1^d$ $n = 1^e$</td>
</tr>
<tr>
<td></td>
<td>4–16</td>
<td>3–29</td>
<td>3–1 3–4 10–16 7–19 17 29 17</td>
</tr>
<tr>
<td>Solids</td>
<td>–</td>
<td>45.8</td>
<td>– 48.8$^{d}$ 43.8$^g$ 47.6 46.7 43.9 44.1</td>
</tr>
<tr>
<td>Protein</td>
<td>11.5</td>
<td>10.8</td>
<td>– 9.8 11.5 10.9 12.6 10.3 10.2</td>
</tr>
<tr>
<td>Casein</td>
<td>–</td>
<td>7.1</td>
<td>– – – 7.1 – – –</td>
</tr>
<tr>
<td>Whey</td>
<td>–</td>
<td>3.8</td>
<td>– – – 3.8 – – –</td>
</tr>
<tr>
<td>Total lipids (fat)</td>
<td>27.2</td>
<td>31.9</td>
<td>– 34.8$^h$ 29.7$^i$ 33.1 32.0 31.0 31.1</td>
</tr>
<tr>
<td>SAFA % of total fat</td>
<td>23.6</td>
<td>39.5</td>
<td>– – – 37.8 39.4 41.3</td>
</tr>
<tr>
<td>MUFA % total fat</td>
<td>43.8</td>
<td>55.2</td>
<td>– – – 55.2 51.7 58.7</td>
</tr>
<tr>
<td>PUFA % total fat</td>
<td>23.4</td>
<td>4.7</td>
<td>– – – 6.3 5.5 2.2</td>
</tr>
<tr>
<td>Ash</td>
<td>–</td>
<td>1.0</td>
<td>– – – 1.4 1.3 0.1 1.1</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>4.6</td>
<td>1.4</td>
<td>– 4.1 1.6$^j$ 0.3 0.6 1.1 0.5</td>
</tr>
<tr>
<td>Gross energy (kJ/g)</td>
<td>–</td>
<td>15.0</td>
<td>– 16.0$^f$ 15.0$^g$ – – – –</td>
</tr>
<tr>
<td>Vitamin D$_3$ (ng/ml, IU/l)</td>
<td>15.4, 650.0$^k$</td>
<td>–</td>
<td>1.62 – – – – – –</td>
</tr>
<tr>
<td>25(OH)D$_3$ (pg/ml, IU/l)</td>
<td>174.3, 35.0$^k$</td>
<td>–</td>
<td>– – – – – – – –</td>
</tr>
<tr>
<td>Vitamin A (µg/ml, IU/l)</td>
<td>0.05, 165.0$^k$</td>
<td>–</td>
<td>– – – – – – – –</td>
</tr>
<tr>
<td>Vitamin E (µg/ml, IU/l)</td>
<td>16.5, 23.8.7$^k$</td>
<td>–</td>
<td>– – – – – – – –</td>
</tr>
<tr>
<td>Taurine (nmol/ml, mg/l)</td>
<td>3165.9, 395.7</td>
<td>–</td>
<td>– – – – – – – –</td>
</tr>
</tbody>
</table>

SAFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids.

$^a$Kenny et al. [1998].
$^b$Derocher et al. [1993a].
$^c$Jenness et al. [1972].
$^d$Cook et al. [1970].
$^e$Baker et al. [1963].
$^{f}n = 39$.
$^{g}n = 49$.
$^{h}n = 41$.
$^{i}n = 65$.
$^{j}n = 59$.
$^{k}n = 14$. 
Protein and Amino Acids

Total protein concentration of the samples analyzed was similar to published data and early lactation milk did not differ statistically in protein content from mid-lactation milk ($t$-test, $t = 0.35$, df = 14, $P = 0.73$).

Twenty-four amino acids were measured in milk samples (Fig. 2). Taurine was in the highest concentration followed by ornithine. Comparing taurine concentration levels in more traditional mg/l units (Table 3) with assorted species shows similar

Fig. 1. Average comparative distribution of fatty acids in milk from free-ranging polar bears from Svalbard, Norway. Early stage of lactation (cubs <4 months of age).1 Mid-lactation (yearlings >16 months of age).2 SAFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids. Analyses performed at the University of California, Davis.

Fig. 2. Mean milk (SEM) free amino acid values from free-ranging polar bears from Svalbard, Norway ($n=14$ except where noted). Analyses performed at the Amino Acid Laboratory, University California, Davis.
concentrations to that in the domestic cat and dog. In contrast, the amino acid in lowest concentration was asparagine, followed by ethanolamine and 3-methylhistidine. There were no significant differences in taurine concentrations between the lactation stages (\(t\)-test, \(t = 1.33\), df = 14, \(P = 0.20\)).

**DISCUSSION**

Our results for the composition of total fat, carbohydrate, and protein in polar bear milk were similar to those of Derocher et al. [1993a]. Our analyses provide new insights on fatty acid composition, fat-soluble vitamins, amino acids, using state-of-the-art analyses.

Polar bears are carnivores that primarily consume seals, but occasionally eat plants [Russell, 1975; Derocher et al., 1993b]. Using fatty acid signature analysis, Thiemann et al. [2008] found that the polar bear diet was dominated by ringed seals, although regional variation in other marine mammal prey was evident. Harp seals are not a major prey species in the Svalbard area because the seals are only available in summer when they migrate northwards [Derocher et al., 2002].

The major lipid component of milk of all species is triglyceride, accounting for >95% of the lipid [Mendelson et al., 1977]. The composition of triacylglycerols is presented as the type and amounts of fatty acids. In carnivores and other simple-stomached animals, the fatty acids deposited in membrane phospholipids and storage triacylglycerols change little from those consumed, so the fatty acid composition of adipose tissue reflects that of the diet over the previous weeks or months [Pond and Ramsay, 1992]. Similarly, the fatty acid composition of

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**TABLE 3. Taurine Content of Milk From Various Species (as-fed basis)**

<table>
<thead>
<tr>
<th>Species</th>
<th>Reference</th>
<th>Stage of lactation after birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baboon, <em>Papio</em> sp.</td>
<td>2, 3</td>
<td>47.5</td>
</tr>
<tr>
<td>Cat, domestic, <em>Felis catus</em></td>
<td>2, 3</td>
<td>350.0 360.0</td>
</tr>
<tr>
<td>Chimpanzee, <em>Pan troglodytes</em></td>
<td>2, 3</td>
<td>88.7 32.5</td>
</tr>
<tr>
<td>Cow, <em>Bos</em></td>
<td>2, 3</td>
<td>38.7 1.3</td>
</tr>
<tr>
<td>Dog, <em>Canis lupus familiaris</em></td>
<td>2, 3</td>
<td>330.0 238.7</td>
</tr>
<tr>
<td>Fishing cat, <em>Prionailurus viverrinus</em></td>
<td>4</td>
<td>58.4</td>
</tr>
<tr>
<td>Gerbil, <em>Gerbillus</em> sp.</td>
<td>2, 3</td>
<td>744.5</td>
</tr>
<tr>
<td>Guinea pig, <em>Cavia porcellus</em></td>
<td>2, 3</td>
<td>21.2</td>
</tr>
<tr>
<td>Horse, <em>Equus caballus</em></td>
<td>2, 3</td>
<td>3.8</td>
</tr>
<tr>
<td>Java monkey, <em>Macaca fascicularis</em></td>
<td>2, 3</td>
<td>17.5</td>
</tr>
<tr>
<td>Lion, <em>Panthera leo</em></td>
<td>4</td>
<td>165</td>
</tr>
<tr>
<td>Man, <em>Homo sapiens sapiens</em></td>
<td>2, 3</td>
<td>51.3 42.5</td>
</tr>
<tr>
<td>Mouse, <em>Mus musculus</em></td>
<td>2, 3</td>
<td>93.8</td>
</tr>
<tr>
<td>Polar bear, <em>Ursus maritimus</em></td>
<td>This survey(^a)</td>
<td>395.7</td>
</tr>
<tr>
<td>Rabbit, <em>Oryctolagus cuniculus</em></td>
<td>2, 3</td>
<td>17.5</td>
</tr>
<tr>
<td>Rat, <em>Rattus norvegicus</em></td>
<td>2, 3</td>
<td>78.8 18.7</td>
</tr>
<tr>
<td>Rhesus monkey, <em>Macaca mulatta</em></td>
<td>2, 3</td>
<td>76.3 70.0</td>
</tr>
<tr>
<td>Sheep, <em>Ovis aries</em></td>
<td>2, 3</td>
<td>85.0 17.5</td>
</tr>
<tr>
<td>Water buffalo, <em>Bubalus bubalus</em></td>
<td>2</td>
<td>23.7</td>
</tr>
</tbody>
</table>

\(^{a}\) Analyzed by the Amino Acid Laboratory at the University of California, Davis.
triacylglycerols in polar bear adipose tissue is reflected in the fatty acid composition of milk [Pond et al., 1992]. In contrast, the fatty acid composition of ruminant (e.g. cattle) adipose tissue [St John et al., 1987] and milk [Avila et al., 2000; DePeters et al., 2001] does not reflect the fatty acid composition of the diet. In ruminants, biohydrogenation of unsaturated fatty acids occurs in the reticulo-rumen compartment of the stomach [Harfoot, 1978]. In the biohydrogenation pathway, there are \textit{trans} fatty acid intermediates. Because biohydrogenation of all PUFA, including linoleic and linolenic acids, to stearic acid is incomplete, these \textit{trans} fatty acids can be used for lipid synthesis. Two important intermediates of rumen biohydrogenation are C18:1 \textit{trans} 11 (\textit{trans} vaccenic acid) and C18:2 \textit{cis} 9 \textit{trans} 11 (rumenic acid). The latter is a conjugated linoleic acid isomer found in ruminant lipids but not typically found in the lipid of non-ruminants and plants [Chin et al., 1992]. C18:1 \textit{trans} 11 and C18:2 \textit{cis} 9 \textit{trans} 11 in the milk lipid of two polar bears leads us to suspect that these bears had or were consuming reindeer.

The milk fat of carnivores is characterized mainly by C14:0–18:2 [Iverson and Oftedal, 1995]. Our results were similar to carnivore and cetacean values compiled by Iverson and Oftedal 1995. Our fatty acid data for 14 of the 16 bears studied were also similar to milk values of aquatic mammals that consume marine lipid containing large amounts of C16:1 [Iverson and Oftedal, 1995] and other long-chain unsaturated fatty acids [Mendelson et al., 1977]. However, two bears were distinctly different in fatty acid profile from the other 14 polar bears, indicating a difference in diet. The milk lipids of two polar bears contained C18:2 \textit{cis} 9 \textit{trans} 11 (Fig. 3),

![Fig. 3. Mean selected fatty acids (SEM) in milk of two polar bears suspected of consuming reindeer as part of their diet compared with 14 bears consuming a marine diet. C22:6n3, docohexaenoic acid; C20:5n3, eicosapentaenoic acid; C20:4n6, arachidonic acid; C18:2 \textit{cis} 9 \textit{trans} 11, rumenic acid; C18:1 \textit{trans} 11, \textit{trans} vaccenic acid; SCFA, short-chain fatty acids; MCFA, medium-chain fatty acids; SCFA, sum of (C4:0+C6:0+C8:0); MCFA, sum of (C9:0+C10:0+C11:0+C12:0+C13:0+C14:0).](image)
whereas this fatty acid was not found in the milk lipids of the other 14 polar bears. These two polar bears also had C18:1 \textit{trans} 11 in their milk lipids (Fig. 3). The fatty acid composition of these two bears was lower in the long-chain, polyunsaturated n3 fatty acids (C20:5 n3 and C22:6 n3) and higher in C20:4 n6, short-chain fatty acids (SCFA), and medium-chain fatty acids. C16:0 and C18:0 were higher and mono-unsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were in lower concentration for these two bears compared with the other 14 bears (Fig. 4). We believe that these two bears were consuming a diet that was different from the diet consumed by the other 14 bears at the time milk samples were collected and this was supported by the capture of both bears near reindeer carcasses. Reindeer are often scavenged by polar bears in Svalbard [Derocher et al., 2000]. Thiemann [2008] proposed that the fatty acid signature of adipose tissue from polar bears that included \textit{trans} may indicate ungulates in the diet. The relationship between diet and tissue composition is different in ruminants such as reindeer. Microbes in the rumen convert much of the dietary carbohydrates to short-chain fatty acids, and alter the structure of the plant lipids. The reindeer’s liver and adipocytes also synthesize long-chain fatty acids from short-chain fatty acids [Larsen et al., 1985].

Previous work from Hedberg et al. [2006] compared the fatty acid distribution of liquid Esbilac\textsuperscript{®} (Pet Ag Inc., Hampshire, IL 60140), a common milk replacer to published milk data, adipose tissue, and selected plant and fish oils. The authors

![Fig. 4. Mean selected saturated fatty acids and total unsaturated fatty acids (SEM) in milk of two polar bears suspected of consuming reindeer as part of their diet compared with 14 bears consuming a marine diet. PUFA, polyunsaturated fatty acids; MUFA, mono-unsaturated fatty acids; C18:0, stearic acid; C16:0, palmitic acid; PUFA, sum of (C18:2+C18:3n6+C18:3n3+C20:2+C20:3+C20:4+C20:5n3+C22:2n6+C22:3n3+C22:4n6+C22:5n6+C22:5n3+C22:6); MUFA, sum of (C14:1 \textit{cis}+C15:1 \textit{cis}+C16:1 \textit{cis}+C17:1 \textit{cis}+C18:1 \textit{cis} 9&10+C18:1 \textit{cis} 11+C18:1 \textit{cis} 12+C18:1 \textit{cis} 13+C22:1+C24:1).]
concluded that more investigation was needed on the specific milk FA composition. The fatty acid composition of milk formula for hand-reared polar bear cubs should have similar concentrations of the reported fatty acids in milk from our study. For many mammals, C18:2 n6 and C18:3 n3 are considered as dietary essential fatty acids; linoleic acid (C18:2 n6) can be converted to arachidonic acid (C20:4 n6). However, the domestic cat is believed to require a dietary source of C20:4 n6 [Morris, 2002] as a consequence of what the author referred to as “idiiosyncratic nutritional requirements” of selected nutrients associated with “activities of enzymes involved in the metabolic pathways of these nutrients.” Morris [2002] also suggested that cats might have a dietary need for omega-3 PUFA. It is unknown whether polar bears require a dietary source of C20:4 n6 or omega-3 FA; for example, C18:3n3, C20:5n3, and C22:6n3.

Limited placental transfer of fat-soluble vitamins occurs in humans, rats, and lambs [McDowell, 2000; Martin and Hurley, 1977; Njeru et al., 1994]. Variations occur in the concentration of fat-soluble vitamins in milk in different species [Csapó et al., 1996]. Variation in vitamins is also evident between colostrum (first milk produced by the mammary gland) and subsequent milk produced by terrestrial animals [Gay and Besser, 1991; Boersma et al., 1991] and phocids [Schweigert and Stobo, 1994]. In human milk, both vitamin A and E concentrations decreased from colostrum values through lactation [Macias and Schweigert, 2001]. Queens’ milk as analyzed by Dobenecker et al. [1998] showed a decrease in vitamin A from $2.2 \mu g/ml \pm 0.7$ at week 1 to $1.57 \mu g/ml \pm 0.8$ at 4 weeks. Several reports on phocid seals [Debier et al., 1999; Schweigert and Stobo, 1994] with short lactation periods found that colostrum contained significantly higher amounts of vitamin E compared with milk produced later in lactation, whereas the vitamin A content remained unchanged. Although this study did not measure colostrum samples, there was temporal variation in vitamin A and E with higher levels in early lactation compared with mid-lactation.

The vitamin D content of milk is generally expressed as IU/l because this nomenclature is employed in the supplementation of cows’ milk [Reeve et al., 1982a; Hollis and Wagner, 2004]. Polar bear milk has a high concentration of vitamin D$_3$ (15.4±3.8 ng/ml or 650±152 IU/l) compared with unsupplemented cows’ milk (1.0 ng/ml or 40 IU/l) and human milk (40–50 IU/l) [Reeve et al., 1982a,b; Hollis and Wagner, 2004]. Thus, the average concentration of vitamin D$_3$ in polar bear milk is 15-fold higher than in cows or humans, and more than 1.5 times higher than in supplemented cows’ milk. Even the lowest concentration measured in our study (3.1 ng/ml, or 149 IU/l, bear #15) is nearly 4-fold higher than the average for the other two species.

Vitamin D is insoluble in the aqueous phase of milk and is found in the lipids. Because of the high fat content of polar bear milk (average 28.7%), and because of the marine mammal diet of polar bears, high vitamin D content is not surprising. However, there was no correlation between the fat content of a milk sample and the vitamin D concentration.

Vitamin D is a conditionally essential nutrient for most animals because when skin is exposed to ultraviolet light from the sun the skin converts 7-dehydrocholesterol to cholecalciferol vitamin D$_3$ [Morris 2002]. Cats have an obligatory dietary requirements for vitamin D as their skin contains a low concentration of 7-dehydrocholesterol, the precursor for pre-vitamin D [How et al., 1994; Morris
et al., 1999]. Study by Kenny et al., in skin samples of six captive polar bears having low levels of 7-dehydrocholesterol, suggests that ingestion of vitamin D is more important for polar bears than cutaneous synthesis [Kenny et al., 1998]. If so, polar bears have an obligate dietary need for vitamin D, and vitamin D concentrations in milk or milk formula will be important for developing young.

Taurine is a major constituent in the milk of many mammals and is the free amino acid present in greatest concentration in man [Verner et al., 2007], cetaceans [Walsh and Rogers, 1995] dogs, cats, gerbils, rhesus monkeys and is exceeded by only one amino acid in rats, baboons, and chimpanzees [Sturman, 1986]. The concentrations of the dominant free amino acid measured in this study, taurine, suggest that hand-reared polar bear cubs fed an artificial milk replacer would benefit from added taurine. Tauro-conjugated bile salts have synergistic properties to improve fat and fat-soluble vitamin digestion and absorption [Stamp and Jenkins, 2008]. The results are also important when compared with other species, as noted in Table 3. Studies on kitten and infant primate development suggest that taurine is an important amino acid for the prevention of retinal degeneration, cardiomyopathy, and abnormal growth and development [Hayes et al., 1980; Hayes and Trautwein, 1989].

Our results have implications to minimize nutritional developmental problems in polar bear cubs associated with inadequate nutrients. This study provides valuable information for the dietary needs of neonates and yearlings to prevent the onset of metabolic bone disease.

CONCLUSIONS

1. We report analyses of the nutrient composition of milk obtained from 16 lactating polar bears with cubs. Comparisons between early and mid-lactation milk samples confirm that milk composition for fat, fatty acids, carbohydrate, fat-soluble vitamins, protein, and taurine did not vary substantially. Our results for total fat, carbohydrate, and protein were similar to early and mid-lactation samples as reported by Derocher et al. [1993a]. New data are presented for amino acids and fat-soluble vitamins D₃ and 25(OH)D₃ that have not been previously reported. The ranges in concentration for various nutrients are wide and likely to reflect environmental factors including diet, stage of lactation, nursing frequency, and sample collection parity.

2. Our comprehensive results give an accurate summary using modern analytical methods for free-ranging polar bears on a natural diet.

3. Our data for fatty acid distribution for SAFA, MUFA, and PUFA are helpful because it is presented as percent of the total fat in maternal milk. The influence of diet was reflected in selected fatty acids absent in the milk of 14 polar bears but present in the milk of 2 polar bears. These fatty acids, C18:1 trans 11, and C18:2 cis 9 trans 11, are intermediates of rumen biohydrogenation of PUFA and are found in ruminant lipids. The milk was from these two polar bears caught near reindeer carcasses, suggesting a link between diet and milk fatty acid composition.

4. Vitamin D, vitamin A, and omega-3 PUFA showed considerable variation in concentration.
5. A detailed comparison from published polar bear milks from early, mid, and late lactation varied from our samples from early and mid-lactation for carbohydrate and vitamin \( \text{D}_3 \). We present new results for \( 25(\text{OH})\text{D}_3 \), vitamin A, vitamin E, and amino acids.

6. Taurine was the dominant free amino acid and the concentration in polar bear milk was high and may reflect a dietary requirement for this amino acid.

7. Each milk constituent has unique characteristics and biochemical properties that impact absorption and utilization of all the nutrients. Early lactation milk composition information will be useful when formulating hand-rearing milk diets for infant polar bear cubs. Similar information for nutrients in mid-lactation milk samples consumed by yearlings will also be helpful for captive weaning diets.

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