Changes in milk composition during lactation in three species of insectivorous bats

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Abstract. Changes in milk composition are described for three species of free-ranging insectivorous bats (Myotis lucifugus, M. velifer, and Tadarida brasiliensis) from early to mid (peak) lactation. Dry matter and energy concentrations in milk increased from early to mid-lactation. In M. lucifugus and T. brasiliensis, but not M. velifer, these increases were due largely to a rise in fat concentration, since protein and carbohydrate remained relatively constant. Energy content of milk (kJ·g⁻¹) for each species from early through mid-lactation was related to dry matter (DM) as follows: M. lucifugus (y = 0.31 DM – 0.32, r² = 0.68), M. velifer (y = 0.48 DM – 0.58, r² = 0.99), and T. brasiliensis (y = 0.37 DM – 1.51, r² = 0.61). Comparison of the effect of sampling method on milk composition of T. brasiliensis indicated that fat, dry matter, and energy concentrations increased significantly from pre-dawn to prenoon samples. Relatively high fat and low water levels in T. brasiliensis milk may reflect the limited access that lactating females have to free water, as well as need to minimize mass of stored milk during long foraging trips. Conversely, lower fat concentrations and higher water levels in milk in M. lucifugus and M. velifer may relate to the propensity for colonies of these two species to roost and forage near bodies of water. In addition, differences in milk fat concentrations observed among the three species may correlate to daily suckling schedules. Females of T. brasiliensis, for example, roost apart from and suckle their young on a regular daily schedule, whereas both species of Myotis roost with their pups and appear to suckle them on demand.

Key words: Milk composition – Lactation – Bats – Free-ranging bats – Microchiroptera

Introduction

As lactation represents the largest energy demand of maternal reproductive effort (Oftedal 1985; Loudon and Racey 1987; Blaxter 1989; Thompson 1992), composition of milk in mammals should be under strong selection pressure. Gross composition and energy concentration of milk can have important implications with respect to water balance and energetics of lactating females and growth and nutrition of developing young. Since lactating females must supply sufficient energy and nutrients to their offspring to support growth to weaning, species producing dilute milks typically deliver large volumes to their young and thereby lose substantial quantities of water (Oftedal et al. 1983; Maltz and Shkolnik 1984; Oftedal 1985). Maternal access to water, distance to foraging sites, frequency of suckling, neonatal body size, maternal body size, lactation length, and the extent to which mothers utilize stored nutrients to support lactation are all thought to correlate to observed phylogenetic trends in milk composition (Ben Shaul 1962; Blaxter 1971; Jenness 1974; Maltz and Shkolnik 1984; Oftedal 1984, 1985; Trillmich and Lechner 1986; Oftedal et al. 1988; Oftedal et al. 1993; Oftedal and Iverson 1995). Unfortunately, many broad phylogenetic comparisons have been flawed by inclusion of suspect data (Oftedal 1984; Oftedal and Iverson 1995).

Relatively little is known about phylogenetic variation in composition of milks of bats, despite the fact that this order encompasses a large number of species representing a range of body sizes (2–1200 g), a diversity of dietary specializations (Hill and Smith 1984), and a wide range of life history characteristics (Kunz 1982). Published data on milk composition are available for only 11 of about 920 species of bats and represent a total of no more than 36 pooled samples (Huibregtse 1966; Jenness 1974; Jenness and Studier 1976; Kunz et al. 1983; Quicke et al. 1984).

Much existing data on bat milks is also flawed by incomplete sampling information. Most samples have been obtained opportunistically from free-ranging bats in...
large colonies and exact stage of lactation has not been known. This may partly explain the large inconsistencies between reports. Does *Myotis lucifugus* milk, for example, contain 6% fat (Jenness and Studier 1976) or more than 13% fat (Kunz et al. 1983)? Does *Tadarida brasiliensis* milk contain 11% protein (Huibregtsen 1966) or about 6% protein (Jenness and Studier 1976)? Although Jenness and Studier (1976) concluded that nectar and fruit-eating phyllostomid bats contain higher carbohydrate and lower protein concentrations than insectivorous species, the small numbers of species sampled and inconsistencies among published reports preclude any definitive life history or phylogenetic analyses.

Our objective was to resolve some of the discrepancies among published reports by more thorough sampling of bats of known lactation stage. Choice of species was also influenced by our interest in the potential constraints on milk composition posed by the dynamics of flight, which places a premium on reduction in wing loading (Norberg and Rayner 1987; Speakman and Racey 1991). Although the high nutrient demands of lactation inevitably require an increase in maternal food intake in small mammals (Kunz 1974; Anthony and Kunz 1977; Randolph et al. 1977; McClure 1987; Thompson 1992; Ofstedal 1993; Kunz et al. 1995), an increase in distance and/or time of foraging tips of bats would entail increased storage of milk between nursing bouts. We predicted that species foraging over great distances and for prolonged periods would produce more concentrated milks to minimize wing loading. We examined this prediction by comparing the milks of two species (*Myotis lucifugus* and *M. velifer*) that forage over relatively short distances with milk of a species (*Tadarida brasiliensis*) that forages over great distances and at relatively high altitudes (Williams et al. 1973; Kunz 1974).

Materials and methods

**Study species.** We examined milk composition in two members of the family Vespertilionidae, the little brown bat (*Myotis lucifugus*), the cave bat (*Myotis velifer*), and one member of the family Molossidae, the Mexican free-tailed bat (*Tadarida brasiliensis*). Each species is relatively small (~7–13 g during lactation) and annually produces a single pup weighing approximately 25–30% of a female’s post-partum bm (Kurta and Kunz 1987). Female *M. lucifugus* and *M. velifer* both roost with their pups during the maternity period and each suckles its own pup on demand (T.H. Kunz, unpublished data). By contrast, female *T. brasiliensis* usually roost separately from their pups during the daytime and suckle their young on a regular diel schedule (McCracken and Gustin 1991).

Roosting conditions and colony size vary markedly among these three species. Maternity colonies of *M. lucifugus* are typically found in man-made structures and range from a few hundred to one thousand individuals (Fenton and Barclay 1980), whereas those of *M. velifer* are found in caves and buildings (Kunz 1973) and typically range from several hundred to thousands of bats. Maternity colonies of *M. lucifugus* and *M. velifer* both are usually located near bodies of water, where bats feed and drink upon departing from their roost at dusk (Kunz 1974; Anthony and Kunz 1977). By contrast, most maternity roosts of *T. brasiliensis* are located in caves where colonies range from several thousand up to 20 million individuals (Davis et al. 1962; McCracken and Gustin 1991). Colonies are most often located in arid and semi-arid regions and, although free-standing water may exist at some locations, individuals from a given colony may disperse nightly up to 50 km where they feed on insects at elevations upwards to 3000 m; they have seldom been observed feeding or drinking over bodies of water (Kunz et al. 1995).

Postnatal growth rates have been estimated and age-predictive equations have been derived for each species (Kunz and Anthony 1982; Kunz and Robson 1995; T.H. Kunz and S.K. Robson, unpublished observations), making possible estimation of day of lactation based on ages of pups at the time mother-pup pairs were captured and milk was sampled.

**Sample collection.** Study sites and dates of sampling for each species were as follows: *M. lucifugus* (June and July 1986, Peterborough, New Hampshire), *M. velifer* (May and June 1988, Eckert James River Cave, Mason County, Texas) and *T. brasiliensis* (June and July 1987, Eckert James River Cave).

Mother-pup pairs were hand-captured at maternity roosts soon after mothers returned from their second (pre-dawn) feeding period and began to suckle their young. Following capture each mother-pup pair was placed in a separate cloth bag and removed from the roost site for processing. Pups were next separated from their mothers and, in each was individually marked on the forearm with a numbered plastic split-ring band (A.C. Hughes). Pups were separated from their mothers by placing a small, tapered metal probe into the corner of the pup’s mouth, at the angle of the jaw, and gently placing pressure at this point until the pup’s mouth opened. Care was taken to avoid damaging fragile deciduous teeth and nipples when pups were removed from mothers. This pup-removal technique was used successfully on all bats without injury to either mother or pup.

Mothers and pups were each weighed separately to the nearest 0.05 g using a portable Ohaus electronic balance and measured. Forearm length was recorded for mothers, and forearm length and epiphysyal gap lengths were recorded for pups (Kunz and Anthony 1982). During the processing period pups and mothers were housed separately in small groups (four to six individuals) inside simulated roosts (Kunz and Kurta 1988) in an effort to minimize water stress and to maintain a thermal and social environment similar to the bat’s natural roost environment. Milk from *Myotis lucifugus* and *M. velifer* was expressed after mothers had been separated from their pups for 4–6 h. Previous studies of *M. lucifugus* showed that bats which suckle on demand produce larger volumes of milk if separated from their pups for this duration (Kunz et al. 1983). Because milk volume was higher in *T. brasiliensis* during mid-lactation, it was possible to milk individuals within 1 h of capture as well as 4–6 h after separation to determine if time of milk collection affected milk composition. After milking, pups were returned to their mothers (matching band numbers). Pairs were released at the site of capture after other adults had departed at dusk on the same day to minimize colony disturbance. Day of lactation for each mother was assigned based on the age of the attached pup, as estimated from forearm length and epiphysyal gap length (Kunz and Robson 1995; T.H. Kunz and S.K. Robson, unpublished observations).

Milk was manually expressed from the mammary glands within 5 min of the female receiving an intraperitoneal injection (2 μl g bm⁻¹) of oxytocin (Sigma, 222 IU ml⁻¹). Before milk was expressed, nipples and surrounding skin and pelage were cleaned with a cotton swab moistened with 70% ethanol to minimize contamination from foreign material and to separate surrounding hair from the nipple so that expressed milk would not adhere to the hair. Milk was alternately expressed from both glands of each bat and collected in microcapillary tubes. A non-heparinized capillary tube attached to a SAFE mouth pipette was used to draw up expressed milk and transfer it to a 0.5-ml Eppendorf tube. In the field, milk samples were temporarily stored on gel coolants in an insulated cooler and subsequently transferred to liquid N₂ or dry ice before being stored in an ultracold freezer (~80°C) prior to laboratory analysis.

Tₛ was recorded at roost sites in Eckert James River Cave using a Serdes Hygrothermograph; these data were summarized as daily maximum and minimum temperatures.
Laboratory analysis. Samples were pooled according to day of lactation as described earlier. Approximate volumes were determined by weighing tubes with milk and subtracting the mean mass of empty Eppendorf tubes. Because minimum volume of milk required for duplicate analyses (ca. 0.4 ml) was greater than that produced by a single bat, milk samples were pooled from individuals over a range of ages to achieve this volume (Table 1). For *M. luciferus* and *M. velifer*, we combined milk from a maximum of 17 different females into a single pool, especially in early lactation when milk volume was relatively low. For *T. brasiliensis*, milk from 5–7 females was usually needed for analysis. Pooled samples were vortexed immediately before sub-sampling for each separate analysis to ensure adequate mixing.

Dry matter content of milk was determined by mass change after drying subsamples in a forced convection oven at 100 °C for 3.5 h. Fat was determined by the Roe & Gotschlich method (AOAC 1990) which was modified for small milk samples. Following disruption of fat globule membranes with ammonium hydroxide and ethyl alcohol, each sample was extracted three times with ethyl and petroleum ether. Fat was calculated as the mass loss of the collection pan after the extracted, dried lipid residue was solubilized with warm petroleum ether. Nitrogen content was determined by the Nessler's procedure (Koch and McMeekin 1924) in which samples were digested with sulphuric acid and hydrogen peroxide. Nessler's reagent was added and color development measured and compared to ammonium sulfate standards using a UV-visible spectrophotometer (Milton Roy Spectronic 1201). When applied to cow's milk, this procedure produced similar results as a macro-Kjeldahl procedure; we found no evidence that direct Nesslerization of sulphuric acid digests overestimated N content as suggested by Quicke et al. (1984). Crude protein was calculated as total N × 6.28. Carbohydrate content was determined by the phenol-sulphuric colorimetric method (Marier and Boulet 1959), modified by diluting the phenol to 11% prior to addition to sample tubes and adjusting the relative proportions of reagents accordingly. This improved accuracy of phenol delivery to sample tubes and improved repeatability, given the sensitivity of this assay to the amount of phenol added (Dubois et al. 1956; Marier and Boulet 1959). Standards were prepared with lactose monohydrate and analyzed concurrently.

Energy density of milk was derived by multiplying published energy equivalents (kJ/g DM⁻¹) of fat (38.12), protein (18.52), and carbohydrate (23.51), by the value obtained from proximate analysis (Perrin 1958; Offedal 1984).

Definitions and data analysis. We have defined early, mid- (peak) and late lactation based on relative rates of growth and degree of pup independence (Fig. 1). Early lactation is defined as the period of positive linear growth in bm prior to initiation of flight, mid-lactation as the period when growth rate begins to decline and flight is initiated but pups have not begun to forage, and late lactation as the period when pups have begun to forage but continue to suckle from their mothers. During the latter period, pup mass gain is typically negligible (or mass may be lost). Since we could not consistently capture mother-pup pairs during late lactation, our analysis is based only on milk collected during early and mid-lactation.

We used least-squares linear regression analysis to test the influence of lactation stage on the proximate composition of milk from each species, a single model test of coincidence to examine interspecific differences in these regression equations, and a repeated measures ANOVA to test for diurnal changes in the milk composition of *T. brasiliensis* (Sokal and Rohlf 1969; Cody and Smith 1987; Kleinbaum et al. 1988). Because these analyses represent a series of simultaneous tests, each of which could lead to the acceptance of significant lactational stage or diurnal effects on milk composition, we used a sequential Bonferroni analysis (Rice 1989) to adjust the significance of each analysis. All tests were carried out using SAS (Version 6.07), and were based on the number of pooled samples (N) rather than the total number of individuals that comprised each sample (n). A non-parametric Wilcoxon test was used to test for differences in mean cave temperatures during the lactation period of *M. velifer* and *T. brasiliensis* at the Eckert James River Cave.

Results

Pup growth curves and demarcation of early, mid- and late-lactation stages are shown for each species in Fig. 1. Mid-lactation, as defined here, occurs at an earlier stage of growth and occupies a shorter time period in *M. luciferus* than it does in *M. velifer* or *T. brasiliensis*, but pup growth rates are similar. A more detailed analysis of pup growth in the two latter species is presented elsewhere (Kunz and Robson 1994; T.H. Kunz and S.K. Robson unpublished). Mean age, age range, number of individuals milked and number of pooled samples assayed at each lactation stage are summarized in Table 1. We had to combine milk samples from a relatively large number of individuals over a range of pup ages to obtain sufficient volumes for proximate analysis. During early lactation (days 1–12) milk samples from 60 separate *M. luciferus* females were required to obtain four analyses representing two age ranges (1–6 and 7–12 days). This level of pooling from so many females and pup ages clearly limits
Table 1. Summary of milk composition during early and mid-lactation in Myotis lucifugus, M. velifer, and T. brasiliensis

<table>
<thead>
<tr>
<th>Mean age</th>
<th>n</th>
<th>N</th>
<th>Milk composition (X ± SD)</th>
<th>Energy (kJ g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fat</td>
<td>Protein</td>
</tr>
<tr>
<td>Myotis lucifugus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.8 (1-6)</td>
<td>34</td>
<td>2</td>
<td>10.88 ± 0.36</td>
<td>9.65 ± 1.24</td>
</tr>
<tr>
<td>9.8 (7-12)</td>
<td>26</td>
<td>2</td>
<td>13.92 ± 1.06</td>
<td>9.26 ± 0.50</td>
</tr>
<tr>
<td>X early lactation</td>
<td>60</td>
<td>4</td>
<td>12.40 ± 1.87</td>
<td>9.45 ± 0.80</td>
</tr>
<tr>
<td>16.2 (13-19)</td>
<td>30</td>
<td>3</td>
<td>14.06 ± 1.15</td>
<td>8.57 ± 0.07</td>
</tr>
<tr>
<td>22.0 (19+)</td>
<td>11</td>
<td>2</td>
<td>19.19</td>
<td>8.37</td>
</tr>
<tr>
<td>X peak lactation</td>
<td>42</td>
<td>3</td>
<td>15.77 ± 3.07</td>
<td>8.50 ± 0.13</td>
</tr>
</tbody>
</table>

| Myotis velifer |   |   |     |         |             |            |     |          |
| 2.7 (1-3) | 9 | 1 | 7.55 | 9.02 | 3.92 | 22.04 | 77.96 | 5.51 |
| 8.6 (4-12) | 8 | 1 | 8.3 | 9.31 | 4.24 | 22.74 | 77.26 | 5.93 |
| 16.9 (13-19) | 11 | 1 | 18.55 | 9.35 | 3.78 | 31.35 | 68.65 | 9.73 |
| X early lactation | 28 | 3 | 11.57 ± 6.15 | 9.23 ± 0.18 | 3.98 ± 0.24 | 25.38 ± 5.18 | 74.62 ± 5.18 | 7.06 ± 2.32 |
| 21.6 (20-23) | 3 | 1 | 23.89 | 11.15 | 4.19 | 35.78 | 64.23 | 12.20 |
| 27.9 (24-29) | 5 | 1 | 16.94 | 10.75 | 4.40 | 30.32 | 69.68 | 9.53 |
| 31.0 (30-32) | 3 | 1 | 18.71 | 10.05 | 4.54 | 31.08 | 69.92 | 10.17 |
| X peak lactation | 11 | 3 | 19.90 ± 3.59 | 10.65 ± 0.56 | 4.38 ± 0.18 | 32.39 ± 2.95 | 67.94 ± 3.22 | 10.63 ± 1.39 |

| Tadarida brasiliensis |   |   |     |         |             |            |     |          |
| 4.7 (1-7) | 21 | 3 | 17.50 ± 0.45 | 7.87 ± 0.35 | 3.86 ± 0.24 | 28.67 ± 1.04 | 71.33 ± 1.04 | 9.07 ± 0.22 |
| 11.1 (8-14) | 20 | 3 | 16.27 ± 4.10 | 8.43 ± 0.40 | 3.68 ± 0.07 | 29.29 ± 3.79 | 70.71 ± 3.79 | 8.66 ± 1.64 |
| 18.1 (15-21) | 23 | 5 | 17.81 ± 3.19 | 8.55 ± 0.81 | 3.38 ± 0.57 | 29.17 ± 2.99 | 70.83 ± 2.99 | 9.20 ± 1.27 |
| X early lactation | 64 | 11 | 17.30 ± 2.82 | 8.33 ± 0.64 | 3.59 ± 0.44 | 29.07 ± 2.59 | 70.93 ± 2.59 | 9.02 ± 1.12 |
| 25.9 (22-28) | 26 | 8 | 24.27 ± 7.43 | 7.39 ± 0.72 | 3.51 ± 0.41 | 37.02 ± 5.18 | 62.99 ± 5.18 | 11.48 ± 2.72 |
| 32.8 (29-35) | 27 | 6 | 24.07 ± 3.81 | 7.68 ± 0.53 | 3.42 ± 0.50 | 36.12 ± 3.22 | 63.88 ± 3.22 | 11.44 ± 2.12 |
| 38.8 (36-42) | 24 | 7 | 29.06 ± 6.49 | 8.06 ± 0.55 | 3.14 ± 0.53 | 36.26 ± 5.28 | 63.74 ± 5.28 | 13.34 ± 2.47 |
| X peak lactation | 77 | 21 | 25.81 ± 0.41 | 7.69 ± 0.65 | 3.36 ± 0.48 | 36.51 ± 4.53 | 63.49 ± 4.53 | 12.09 ± 2.40 |

Sample sizes used in statistical analysis (N) are based on data pooled from several individuals (n) within a defined age range. Pooled samples during early and mid-lactation stages are based on criteria established for stage of postnatal development (see text).

The ability to detect significant effects of age and other variables on milk composition.

The relationship between milk composition and pup age is illustrated for each species in Figs. 2 and 3. Energy density of milk increased significantly during lactation in Myotis lucifugus and T. brasiliensis (P < 0.05 and P < 0.001, respectively; Fig. 3) due to significant increases in fat concentration (P < 0.01 and P < 0.001); however, the percentage of protein and carbohydrate remained constant during lactation in each species (Fig. 2). Increase in fat concentration also explains the significant increase in percentage DM in T. brasiliensis (P < 0.001), but not in M. lucifugus (P > 0.05, Fig. 3). Although the best fit regression lines for energy and fat concentrations of the milk of M. velifer are positive (Figs. 2, 3), the equations are not quite significant at the 0.05 level (P = 0.07 and P = 0.06). The regression equations, including standard errors for the intercept and regression coefficient, are as follows:

M. lucifugus:

- Dry matter (% of total mass) = 0.20 ± 0.16 (pup age in days) + 24.37 ± 2.07
  \( F = 1.70, df = 6, P = 0.2490 \) (1)
- Milk fat (% of milk by mass) = 0.37 ± 0.09 (pup age in days) + 9.51 ± 1.14
  \( F = 18.54, df = 6, P = 0.0077 \) (2)

M. velifer:

- Energy content (kJ g⁻¹) = 0.13 ± 0.03 (pup age in days) + 6.39 ± 0.46
  \( F = 14.16, df = 6, P = 0.0131 \) (3)

T. brasiliensis:

- Dry matter (% of total mass) = 0.38 ± 0.16 (pup age in days) + 22.06 ± 0.46
  \( F = 5.77, df = 5, P = 0.0742 \) (4)
- Milk fat (% of milk by mass) = 0.45 ± 0.16 (pup age in days) + 7.46 ± 3.25
  \( F = 5.97, df = 5, P = 0.0710 \) (5)
- Energy content (kJ g⁻¹) = 0.19 ± 0.07 (pup age in days) + 5.44 ± 1.49
  \( F = 6.82, df = 5, P = 0.0593 \) (6)
Based on regression analyses, estimated fat concentration of milk at day 1 was 9.9, 7.9, and 13.6% for *M. lucifugus*, *M. velifer*, and *T. brasiliensis*, respectively. By mid- (peak) lactation, predicted fat concentration increased to 15.4% in *M. lucifugus* (day 16), 17.8% in *M. velifer* (day 23), and 23.8% in *T. brasiliensis* (day 28); representing 0.56-, 1.25-, and 0.75-fold increases, respectively. Relationships between percent milk fat versus pup age in *M. lucifugus* and *T. brasiliensis* were not significantly different (test of coincidence, $P > 0.05$).

Energy concentration of milks of *M. lucifugus*, *M. velifer*, and *T. brasiliensis* were significantly correlated with percentage DM (Fig. 4), and these relationships were not significantly different between species (test of coincidence, $P > 0.05$). The equations describing these relationships are as follows:

*M. lucifugus*:

Energy content (kJ·g DM$^{-1}$) = 0.31 ± 0.09 (% dry matter) - 0.32 ± 2.55

($F = 10.52$, $df = 6$, $P = 0.0229$)  

*M. velifer*:

Energy content (kJ·g DM$^{-1}$) = 0.48 ± 0.02 (% dry matter) - 5.08 ± 0.50

($F = 802.68$, $df = 5$, $P < 0.0001$)  

*T. brasiliensis*:

Energy content (kJ·g DM$^{-1}$) = 0.37 ± 0.05 (% dry matter) - 1.51 ± 1.84

($F = 47.66$, $df = 31$, $P < 0.0001$)

The effect of sampling procedure on measured milk composition in *T. brasiliensis* is illustrated in Fig. 5. Each mean is based on six pooled samples ($N$) comprising a total of 30 individual bats ($n$). Because a significant difference in milk concentration would be indicated by changes in either of the three milk components examined (fat, protein, and carbohydrate) we used a sequential Bonferroni test with an initial probability value of 0.05

$$\frac{0.05}{3} = 0.0167$$

to analyze the probabilities derived from Wilcoxon tests (Rice 1989). Percentage DM, water, and total energy could not be included in this ana-
We report changes in the milk composition in the period from early to mid-lactation in three species of free-ranging insectivorous bats (M. lucifugus, M. velifer, and T. brasiliensis). Estimated day of lactation was based on age of pups captured as mother-pup pairs. Because milk volumes were relatively small from each bat (10–300 μl), pooling samples from many same-aged individuals was necessary for proximate analysis. Regression analyses of these pooled samples indicate that dm and energy concentrations increased as lactation progressed in M. lucifugus and T. brasiliensis, largely due to increases in fat concentration; carbohydrate and protein levels remained relatively constant from early to mid-lactation. A similar pattern of changes in milk composition may also occur in M. velifer, but our small number of pooled samples in this species (n = 5) led to probabilities of 0.05 < P < 0.10 for these relationships. Based on regression equations, milk energy density at day 1 of lactation was lowest in M. lucifugus (9.9 kJ·g⁻¹) and highest in T. brasiliensis (13.6 kJ·g⁻¹).

Fat concentration (as predicted by regression) increased from ~10 to 18% in M. lucifugus, from ~8 to 20% in M. velifer, and from ~13 to 28% in T. brasiliensis over the course of early to mid-lactation. Previous analyses of milk composition in M. lucifugus indicated fat values of 6% (Jenness and Studier 1976) and 12–14% (Kunz et al. 1983). The value reported by Jenness and Studier (1976) is considerably lower than our results, but the estimates given in Kunz et al. (1983) fall within the range of our values. Sampling and analytical methods are not provided by Jenness and Studier (1976) but may underlie the observed discrepancy from the present study. Samples obtained by Kunz et al. (1983) were obtained from sequential dates of capture during the growth period, and may have included bats from both early and
mid-lactation. The relatively low and stable estimates of carbohydrate and protein concentrations (Fig. 2; Table 1) are consistent with previous estimates for this species (Jenness and Studier 1976; Kunz et al. 1983).

Fat concentration of *T. brasiliensis* milk increased significantly from early to mid lactation, and represents the highest reported value for any bat species, with sample means reaching 29% (Table 1). The only previous estimate of fat level in *T. brasiliensis* milk (16.3%; Jenness and Studier 1976) falls within the range of values observed during early lactation in the present study. The relatively high fat level of *T. brasiliensis* milk is largely responsible for the high DM concentration (Table 1, Fig. 2). Although Huibregtse (1966) did not analyze milk samples for fat content, the value he reported for dry matter (34.4%) falls within the range we observed during mid-lactation (Table 1). Estimates of protein and carbohydrate content observed in the present study are higher than those reported by Jenness and Studier (1976) and may reflect differences in sampling protocols or stage of lactation.

The potential effect of sampling procedure is illustrated by the comparison of milk samples obtained from *T. brasiliensis* shortly after returning from feeding in the predawn period with those obtained after 4–6 h of separation (Fig. 5). We do not know if the observed increase in fat concentration represents a sampling bias, such as could occur if the degree of mammary evacuation differed at the two sampling periods (Oftedal 1984; Oftedal and Ivenson 1995), or a diurnal or stress-related change in the relative rates of secretion of fat globules versus the aqueous phase of milk. An increase in relative fat secretion and consequent decrease in milk water concentration might be of adaptive value for lactating bats that roost with limited access to water. Roosting bats typically lose body water from respiration, evaporation, feces, and urine (Kurta et al. 1989), and milk production would impose an additional drain. Moreover, the *T. c.* in caves occupied by lactating *T. brasiliensis* may excrete respiratory and evaporative water losses.

Water conservation may be of particular importance to *T. brasiliensis* given its life history characteristics. Unlike *M. lucifugus* and *M. velifer*, which typically forage over water and consume emergent insects that are often high in water content (Kunz 1974; Anthony and Kunz 1977), *T. brasiliensis* usually feeds in open areas independent of standing bodies of water and consumes largely moths and ant larvae (Kunz et al. 1995), both of which are rich in fat and relatively low in preformed water (Redford and Dorea 1984; Allen 1989). In contrast to the two species of *Myotis* which have been observed drinking at foraging sites (Kunz 1974; Anthony and Kunz 1977), there are few reports of *T. brasiliensis* either flying, feeding or drinking near bodies of water (Kunz et al. 1995). Gelsos (1980) reviewed studies which indicate that *T. brasiliensis* has evolved renal adaptations to reduce water loss. Higher fat concentration, and lower water levels in the milk of this species, as compared to the two *Myotis* species, may be a further adaptation to minimize water loss. Higher energy density of *Tadarida* milk also implies lower milk output in that pups will not need to consume as much milk to meet their energy requirements as they would if milk were more dilute.

This explanation of the difference in milk composition among the bat species studied, while attractive, has little precedence in other mammalian groups. Although Kooyman (1963) claimed that desert-adapted kangaroo rats produced exceptionally high fat milk, the high fat levels observed are typical of many small rodents and not just those adapted to arid environments (Baverstock et al. 1976; Oftedal 1984). Similarly, Maltz and Schkolnik (1984) noted that the milks of desert gazelles and ibex were considerably lower in water than milk of goats, but DM concentrations of gazelle and ibex milks resemble most other wild ungulates (Oftedal 1985; Robbins et al. 1987). Perhaps the best case that high fat and low water levels in milk represent water conservation is that of bears, phocid seals and baleen whales that fast during much or all of lactation (Oftedal 1993).

Many large mammals adapted to hot arid conditions, such as rhinos, equids, and giraffes, produce relatively dilute milks, presumably because of water needs of young for evaporative cooling (Oftedal and Ivenson 1995). The neonates of small terrestrial mammals typically escape extremes of temperature and relative humidity by remaining in burrows or caves. Even if maternal milk is low in water, its intake by suckling young is augmented by metabolic water. Each gram of fat that is oxidized, for example, yields about 1.0 g water as compared to about 0.40 g and 0.56 g water per g carbohydrate and protein, respectively (Brody 1945); water yield per kJ energy released is similar for all three substrates. In many rodents and carnivores, non-evaporative water losses of young are largely recovered by the mother by ingestion of urine and feces of the young (Baverstock and Green 1975); in denning bears the amount so recovered may equal water output in milk since much metabolic water is also recovered (Oftedal et al. 1993). Although no data are available, lactating bats in arid regions might recycle water in this fashion, especially in a species such as *T. brasiliensis*, where water conservation appears to be particularly important.

An alternative explanation for the higher concentration of *T. brasiliensis* milk is that high fat and energy concentrations represents a means of reducing the mass or volume of milk that must be stored and transported by bats that forage over great distances. Since mammary storage volume is finite, and increased mass of filled mammary glands increases wing-loading, there may be a limit to the amount of milk that can be secreted and stored in the mammary glands without adverse effects. If so, amounts of energy, protein and other nutrients transported per foraging bout may depend on their concentrations in milk. One would predict higher energy and nutrient concentrations in milks of bats with fewer, but longer, foraging trips. The first nightly foraging bout averages 5.3 h for a lactating female *T. brasiliensis* (Kunz et al. 1995), ~2 h for *M. lucifugus* (Anthony and Kunz 1977; Kurta et al. 1989a, 1990) and ~2 h for *M. velifer* (Kunz 1974). A similar argument has been put forward to explain differences in milk fat and energy concentrations among species of sea lions and fur seals with different
foraging patterns [Trillmich and Lechner (1986); but see 
Oftedal et al. (1987) for a critique of the data used]. Mamm-
als which feed their young at widely spaced intervals 
appear to produce concentrated, high-fat milk [e.g., rab-
bbits and hares, echidnas, tree shrews and otariid seals: 
Coates et al. (1964); Martin (1968); Griffiths et al. (1984); 
Liulillery et al. (1984); Oftedal et al. 1987)]. Although the 
period of absence may be relatively short for T. brasilien-
sis compared to some of these other species (hours vs. one 
or more days), foraging bouts are considerably longer 
than for other bat species that have been examined. 
Given higher mass-specific rates of milk secretion in 
small mammals (Oftedal 1984), several hours of absence 
in bat-sized mammals may be as important as longer 
time periods in larger mammals.

The higher energy density of T. brasilensis milk may 
also reduce need for frequent suckling when the mother is 
at the roost. Ben Shaul (1962) postulated a correlation 
between suckling behavior and milk composition: spe-
cies in which females suckle their pups on demand were 
thought to produce less concentrated milks than those in 
which suckling occurs on schedule. Although the data 
base included by Ben Shaul (1962) is not reliable (Oftedal 
1984), the three bat species studied herein conform to 
Ben Shaul's hypothesis. T. brasilensis females, which produce 
the most energy-rich milk of the three species, roost apart 
from their pups during the day but suckle their pups at 
least twice daily, first as they return from their evening 
feeding period around midnight and again following 
their return to their roost in the predawn period (Kunz 
et al. 1995). By contrast, female Myotis roost continually 
with their pups during the day and appear to suckle them 
on demand.

In conclusion, unusually high fat and energy concen-
trations, and the relatively low water levels of T. brasilien-
sis milk may relate to several interrelated aspects of the 
life history of this species: arid-adaptation, diet, foraging 
flight length, and suckling pattern. Milk composition and 
lactation strategies of other bats are also likely adapted 
to life history patterns. Compared to other mammals 
for which milk compositions are available (Oftedal and Iverson 
1995), bats are significantly underrepresented as a 
group. Because small volumes of milk must be pooled for 
a complete analysis, data for other species will likely 
accumulate slowly. Notwithstanding practical and analyti-
cal limitations, lactation in bats is especially interesting in 
light of the constraints imposed by flight, which is not 
only energetically expensive but also places a premium 
on mass reduction. Production and transport of high fat 
milk may be an important strategy to minimize the cost of 
flight and at the same time maximize energy transfer to 

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