# Developmental Changes in Composition of Cats' Milk: Trace Elements, Minerals, Protein, Carbohydrate and Fat[1]

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ABSTRACT The concentrations of iron, copper, zinc, manganese, calcium, magnesium, protein, carbohydrate and fat were analyzed in cats' milk during the course of lactation. Cats' milk is different from most species in that the concentrations of iron, copper, zinc and manganese are lower during the first 2 days of lactation than on days 3–7. After the initial rise in the concentration of these elements, the concentration of iron decreased from 5.9 μg/ml to 3.0 μg/ml, with most of the decrease occurring between days 8 and 21. Copper concentration declined from 1.6 μg/ml to 0.8 μg/ml, with most of the change occurring between days 8 and 28. Concentration of manganese increased during lactation, from 0.14 μg/ml to 0.39 μg/ml. Calcium concentration increased rapidly during the first 3 weeks from 550 μg/ml to 1500 μg/ml, with little change thereafter. The magnesium concentration (≃100 μg/ml) and zinc concentration (≃6 μg/ml) were not affected by stage of lactation. Protein increased during lactation from 4% to 7%, and fat from 3% to 5%, whereas carbohydrate concentration (≃4%) did not change significantly. These data demonstrate that the nutrient intake of the kitten changes markedly during the early neonatal period and that these changes should be taken into account in evaluating studies of suckling cats. J. Nutr. *112:* 1763–1769, 1982.

INDEXING KEY WORDS lactation · cats' milk · trace elements · minerals · milk

The developing cat is a potential model for several types of nutritional, physiological and biochemical studies. The early neonatal period is characterized by rapid changes in body composition. To understand the origins and significance of these changes in body composition, it is essential to obtain detailed knowledge of the composition of cats' milk and its variation during the course of lactation, as this is the only source of nutrients during the neonatal period. In addition such information is necessary for the formulation of milk substitutes for kittens.

Although there is some information in the literature with regard to the major nutrients (protein, fat, carbohydrate) in cats' milk, very little is known about the quantitative and qualitative requirements of the young cat for trace elements (Fe, Cu, Zn, Mn) and minerals (Ca, Mg). We were able to find one report in which the mineral composition of cats' milk is discussed, although the data in that report was from only two milk samples, both of which were mature milk (1). We have therefore analyzed the developmental

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changes in concentrations of the trace elements iron, copper, zinc, and manganese, as well as the major mineral elements calcium and magnesium in the milk of domestic short-hair cats. We have also determined the concentrations of protein, carbohydrate and fat in milk and the changes in these nutrients during the course of lactation.

## MATERIALS AND METHODS

*Animals.* Milk samples were obtained from clinically healthy specific pathogen–free domestic short-hair cats in the closed colony at the University of California, Davis. Queens were housed as a group in large (2.4 × 3.0 × 2.2 m) cages until the last trimester of pregnancy when they were transferred to individual galvanized iron or stainless-steel cages (0.9 × 0.9 × 0.9 m) and kept there until the kittens were weaned. Queens were fed dry cat food[2] ad libitum during pregnancy and lactation. During the third trimester and lactation, in addition to the dry food, approximately 60 g of a canned food were offered daily.

*Milking procedure.* Lactating cats ($n = 26$) were given 5 IU of oxytocin (intramuscularly) to stimulate milk flow and then milked by gentle hand stripping of the teat. The free-flowing milk was collected in disposable, acid-washed 1 ml pipette tips (West Coast Scientific, Oakland, CA). Collected milk was stored frozen (−5°) in acid-washed plastic vials until analyzed. Analyses were performed on single milk samples. About half of the cats were milked only once. The other cats were milked several times with at least 5 days separating each milking period. A total of 106 samples were collected; in some cases the volume collected was insufficient for all the analyses. Data from cats milked only once were grouped with those from cats milked more than once. Although serial milking may result in changes in milk composition in the rat (2, 3), this was not found to occur in the present study with cats. The day of parturition was designated day 0 of lactation.

*Analytical methods.* Protein was determined in milk samples (50 μl of a 1:30 dilution) by a dye-binding method (Bio-Rad Protein Assay Kit, Richmond, CA) (4). Carbohydrate was determined by the orcinol-sulfuric acid method described by Svennerholm (5), with this technique, the sum of all pentoses and hexoses was estimated. Fat was determined by a calorimetric method by using the sulfuric acid–vanillin reaction described by Zollner and Kirsch (6).

*Atomic absorption spectrophotometry.* Milk samples (300 μl) were wet ashed with 16 *N* nitric acid (2 ml of Ultrex grade, J. T. Baker Co., San Francisco, CA), concentrated by evaporation and diluted with distilled deionized water (7). Calcium, magnesium, iron, copper and zinc were determined by flame atomic absorption spectrophotometry (Perkin-Elmer Model 370, Perkin-Elmer Corp., Norwalk, CT). For magnesium and calcium analysis, the diluted ashed samples were diluted further with 0.1% lanthanum chloride in order to reduce matrix interference (8). Manganese was determined by flameless atomic absorption (Models 157 and 555, Instrumentation Laboratories, Wilmington, MA). Standard addition of known concentration of selected metal to the milk samples indicated recoveries ranging between 98 and 102% for all elements.

*Statistical methods.* Changes in milk composition that occurred throughout the 67 days of the lactation were analyzed by linear regression analysis (Statistical Package for the Social Sciences–subprogram scattergram). For this analysis cat was not included as a fixed independent variable, because milk samples from each animal were not available at all time points. In addition to linear regression, other modes of curve analysis were tested; however, these approaches offered no consistent advantages. The regression equation is shown, along with the $r^2$ and $P$ values, for all analysis. To identify when changes occurred, data were grouped in 7-day periods and analyzed by Student's *t*-test (BMDP/Program P3D) (9). The first week of lactation was further subdivided into days 0–2 and days 3–7 to allow for the very low values observed for some of the constituents during the 0- to 2-day period. Comparisons were

[2] The cats received dry food (Crave, Kal Kan, Vernon, CA) containing: 84 μg Fe; 6 μg Cu; 40 μg Zn; and 14 μg Mn (per gram dry weight) and canned wet food (Kal Kan, Vernon, CA) containing: 318 μg Fe; 23 μg Cu; 64 μg Zn; and 18 μg Mn (per gram dry weight).

TABLE 1

*Composition of cats' milk during lactation*[1]

| Nutrient | Days of lactation 0–2 | 3–7 | 8–14 | 15–21 |
|---|---|---|---|---|
| | | $\mu g/ml$ | | |
| Iron | 4.01 ± 0.48 (6) | 5.93 ± 0.49 (21) | 4.30 ± 0.28$^{2b}$ (17) | 3.87 ± 0.41 (14) |
| Copper | 1.35 ± 0.29 (5) | 1.63 ± 0.13 (21) | 1.34 ± 0.09 (17) | 1.08 ± 0.09 (14) |
| Zinc | 4.66 ± 0.55 (6) | 6.24 ± 0.30$^{a}$ (21) | 6.18 ± 0.39 (17) | 6.70 ± 0.27 (14) |
| Manganese | 0.14 ± 0.04 (7) | 0.18 ± 0.02$^{a}$ (21) | 0.27 ± 0.03 (17) | 0.24 ± 0.02 (14) |
| Calcium | 545 ± 96 (6) | 950 ± 49$^{b}$ (20) | 1,325 ± 79$^{b}$ (15) | 1,520 ± 66 (13) |
| Magnesium | 86 ± 10 (6) | 96 ± 3 (21) | 104 ± 4 (17) | 103 ± 4 (13) |
| | | % | | |
| Protein | 3.97 ± 0.64 (5) | 4.36 ± 0.31 (17) | 4.89 ± 0.25 (15) | 5.53 ± 0.39 (14) |
| Carbohydrate | 3.57 ± 0.15 (6) | 3.69 ± 0.18 (16) | 3.62 ± 0.19 (15) | 3.85 ± 0.18 (14) |
| Fat | 3.39 ± 0.74 (5) | 3.49 ± 0.38 (15) | 3.68 ± 0.42 (15) | 4.80 ± 0.32$^{a}$ (14) |

| Nutrient | Days of lactation 22–28 | 29–35 | 36–42 | 43+ |
|---|---|---|---|---|
| | | $\mu g/ml$ | | |
| Iron | 4.14 ± 0.58 (11) | 3.65 ± 0.35 (11) | 3.02 ± 0.36 (11) | 3.21 ± 0.62 (7) |
| Copper | 0.93 ± 0.12 (11) | 0.91 ± 0.16 (11) | 0.85 ± 0.15 (11) | 0.61 ± 0.12 (7) |
| Zinc | 7.04 ± 0.40 (11) | 5.56 ± 0.26$^{b}$ (11) | 5.86 ± 0.59 (11) | 6.08 ± 0.37 (7) |
| Manganese | 0.30 ± 0.03 (11) | 0.29 ± 0.03 (11) | 0.28 ± 0.05 (11) | 0.39 ± 0.07 (7) |
| Calcium | 1,725 ± 57$^{a}$ (10) | 1,565 ± 62 (10) | 1,570 ± 74 (11) | 1,370 ± 82 (5) |
| Magnesium | 105 ± 7 (10) | 99 ± 5 (10) | 91 ± 9 (11) | 105 ± 9 (5) |
| | | % | | |
| Protein | 6.49 ± 0.56 (11) | 6.16 ± 0.34 (11) | 6.55 ± 0.45 (10) | 7.46 ± 0.41 (6) |
| Carbohydrate | 3.44 ± 0.17 (11) | 3.91 ± 0.15 (11) | 4.10 ± 0.22 (9) | 4.29 ± 0.24 (6) |
| Fat | 5.08 ± 0.28 (11) | 5.86 ± 0.47 (11) | 5.47 ± 0.43 (10) | 5.31 ± 0.91 (6) |

[1] Values are means ± SEM. Number of samples analyzed shown in parentheses. [2] Significantly different from preceding time period [a] ($P \le 0.05$); [b] ($P \le 0.01$).

then made between one time period and the one immediately following.

RESULTS

Iron concentration in cats' milk decreased significantly during the course of lactation (Fe in $\mu$g/ml = 5.33 − 0.05 × days; $r^2 = 0.20$; $P \le 0.001$), from values of 5–6 $\mu$g/ml during the early part of lactation to about 3 $\mu$g/ml in late lactation. An unusual finding was that the colostrum during the first 2 days contained significantly less iron than did milk in the day 2–7 period. Most of the decrease in milk iron concentration occurred during the second week of lactation (table 1).

Copper concentration decreased significantly during the course of lactation (Cu in $\mu$g/ml = 1.57 − 0.02 × days; $r^2 = 0.29$; $P \le 0.001$), from 1.5–3.0 $\mu$g/ml in early lactation to 0.2–1.0 $\mu$g/ml in late lactation. As in the case of iron, the concentration of copper in the colostrum, collected on day 0 of lactation, was lower than milk collected later in lactation. Most of the decline in copper concentration occurred during the weeks 2 and 3 of lactation (table 1).

The zinc concentration of milk did not exhibit a strong developmental pattern, (Zn in $\mu$g/ml = 6.83 − 0.01 × days; $r^2 = 0.01$; $P \le 0.12$). Zinc concentration, similar to that for iron and copper, increased during the first two days of lactation (table 1). However, a trend towards lower levels was observed in late lactation with levels of approximately 6 μg/ml at the end of lactation.

The concentration of manganese in milk significantly increased during the course of lactation (Mn in μg/ml = 0.20 + 0.002 × days; $r^2$ = 0.08; $P \leq 0.002$) from about 0.10 μg/ml on the first day of lactation to more than 0.25 μg/ml after the first week. Most of the increase occured during the first and second weeks of lactation (table 1).

The concentration of calcium in milk significantly increased during the course of lactation (Ca in μg/ml = 203 + 31 × days; $r^2$ = 0.36; $P \leq 0.001$) from slightly above 500 μg/ml in early lactation to more than 1500 μg/ml in late lactation (table 1).

Magnesium concentration was not significantly influenced by the progression of lactation (Mg in μg/ml = 99 + 0.03 × days; $r^2$ = 0.001; $P \leq 0.40$; the average concentration was approximately 90 μg/ml (table 1).

The protein concentration increased steadily during lactation (protein percentage = 4.32 + 0.06 × days; $r^2$ = 0.30; $P \leq 0.001$), from less than 4% during early lactation to about 7% in late lactation (table 1).

Carbohydrate concentration increased slightly during lactation from 3.5 to 4%, however, there was considerable scatter in the data (carbohydrate percentage = 3.59 + 0.008 × days; $r^2$ = 0.036; $P \leq 0.04$) (table 1).

Fat concentration increased significantly during lactation (fat percentage = 3.20 + 0.67 × days; $r^2$ = 0.33, $P \leq 0.001$) from 1.4% to about 5% in later lactation (table 1).

## DISCUSSION

### *Iron*

The iron concentration of cats' milk (approximately 4μg/ml) was found to be considerably higher than that of human milk, which normally ranges from 0.2 to 0.5 μg/ml, and that of milk from most dairy animals, which ranges between 0.2 and 0.3 μg/ml (10). Although it was lower than that reported for some marsupials, the rat, and the dog (approximately 10 μg/ml) (2, 11), the iron concentration of cat milk is similar to that reported for the rabbit (10). An unusual finding in this study was that the colostrum during the first 2 days contained considerably less iron than did milk at the end of the first week of lactation. This observation is in contrast to reports for other species (10). Our observation that the concentration of iron in cats' milk is strongly influenced by the stage of lactation, with values decreasing with time, is similar to the findings for other species (2, 10, 11) including humans (12–15), except for the first 2 days of lactation.

Like the dog, the rat, and some marsupials, the cat is able to secrete milk in which the concentration of iron may be several times higher than that in plasma (normally about 0.5 μg/ml). This finding suggests that transfer of iron into, or retention by, the mammary tissue of the cat occurs through mechanisms different from those in species where milk iron content is equal to or lower than its plasma concentration. Physiological differences in mammary iron uptake between species with iron-rich milk and those with milk low in iron (such as the human) should be elucidated to ascertain whether the cat is a valid model for milk studies from which correlations to humans may be drawn.

It is not known whether the iron in cat milk is absorbed well by the kitten. The infants of other species with iron-rich milk, such as the rat, can absorb almost 100% of the iron ingested (10). Since anemia occurs rarely in young kittens, it has been suggested that the relatively slow growth rate of the kitten compared to the young of other species prevents depletion of its iron stores before consumption of iron-rich solid food begins (16). However, in comparison to the growth rates of other small mammals, that of the kitten is not unusually slow. Therefore, the observation that anemia is found rarely in the kitten may suggest that bioavailability of the milk iron may be high.

### *Copper*

The copper concentration of cats' milk, ranging from approximately 2.0 μg/ml in early milk to 0.5 μg/ml in late milk, is slightly higher than that found for humans (0.3–0.6 μg/ml colostrum, 0.2–0.3 μg/ml mature milk) and most dairy animals (0.1–0.5 μg/ml) (17), but similar to that of the dog (~2.0 μg/ml)

(11), and lower than that of the rat which has a relatively copper-rich milk (3–13 μg/ml) (2). As with iron, the copper concentration of cats' milk was very low in early colostrum as compared to milk 2-3 days postpartum, which is in contrast to reports for other species (17). It has been suggested that colostrum is rich in copper because of a high concentration of protein-bound copper, in particular, immunoglobin-bound copper (18). The cat may be an excellent model for studying this hypothesis by characterizing the protein profile of milk from day 0 to day 5 of lactation.

Except for the first 2 days of lactation, the levels of copper decreased throughout lactation. This type of developmental pattern is similar to that observed for most species (10), although in the dog, concentrations of copper in milk were not influenced by the stage of lactation (11). Further, we have observed that liver copper concentration does not decline in the growing cat (Keen, C. L., unpublished data). This may indicate that the copper from cats' milk is highly bioavailable.

*Zinc*

The concentration of zinc in cats' milk (~6 μg/ml) is similar to that reported for most species. However, in contrast to other species studied, in the cat the milk concentration of zinc was not influenced by the stage of lactation. This may in part be explained by the fact that cats' milk contains a comparatively high proportion of zinc associated to a low-molecular-weight compound (17). The proportion of zinc in milk associated with this fraction in humans does not vary greatly during lactation (17).

*Manganese*

In contrast to several other species (10) cats' milk manganese increases during early lactation. The concentration of manganese in cats' milk is over 10 times higher than that reported in human milk, but is similar to that of the rat, dog, and some ruminants (2, 11, 17). Little is known at this time regarding bioavailability or importance of milk manganese. The recent report by Dupont and coworkers (19), which suggests the occurrence of manganese deficiency in young children with convulsive disorders, demonstrates the need for further studies on milk manganese and its nutritional role.

*Calcium*

Calcium concentrations of cats' milk are similar to those reported for several species. It was strongly influenced by the stage of lactation, increasing over 200% during the first 3 weeks. The pattern of calcium concentration, increasing with lactation time, was similar to that of protein concentration. Since one of the major proteins of feline milk is casein (20), well known for its calcium-binding capacity, the good correlation between calcium and protein concentration is expected.

*Magnesium*

The concentration of magnesium in cats' milk was similar to that reported for other species (2, 11), and was not influenced by the stage of lactation. This observation is similar to reports concerning dog milk and human milk (11, 14), but in contrast to those for cows (21) and rats (2), in which the values decreased with lactation time. Unlike calcium, milk magnesium concentration was not significantly correlated to milk protein concentration. This is in accordance with findings in human milk, where virtually all magnesium is present in a low-molecular-weight fraction and not bound to proteins (22).

*Protein*

The pattern for protein concentration in cats' milk during lactation indicated that protein increases from 2 to 4% in the first part of lactation to about 6–8% by mid- to late lactation. This pattern contrasts that of milk proteins of humans (23) and cows (24), in which the concentration decreases rapidly during the first part of lactation. An increase in milk protein concentration with lactation time has been reported for the rat (2) and dog (11).

The observation that the protein concentration of cats' milk is similar to that of rat and dog was somewhat surprising. It is believed that the cat has a requirement for protein higher than that of most mammalian species, especially considering its relative growth rate. This relatively high requirement is probably the result of an inability of the cat to adapt from an ancestral high "carnivore" protein diet to a low "noncarnivore" protein diet. It has been shown that the activity of the urea cycle enzymes in cat liver are nonadaptive (25). Thus, one might predict that the protein content of cat milk should be high relative to most species. By calculation, the protein of cat milk at midlactation contributes approximately 28% of the total energy. This value is higher than the determined value of the minimal protein requirement for the growth of kittens receiving an "ideal" protein.[3] If cat milk does not exceed the protein requirement of preweaning kittens, the protein requirement of preweaning kittens appears to be higher than that of postweaning kittens. In the adult stage, maintenance protein requirement has been calculated to be about 12% (26). Other species such as the rat and dog have a higher protein requirement during the suckling period than in the postsuckling period. Since the cat has a high requirement for the sulfur containing amino acids, arginine and taurine, the amino acids provided by cat milk may be different from those of most species.

### Carbohydrate

Values obtained in this study for milk carbohydrate (3–5%) are similar to those reported for most species (2, 11). No strong developmental pattern for milk carbohydrate concentration was found, although there was a trend for values to increase with lactation time. A similar weak pattern was found for the dog (11) and the rat (2).

### Fat

An important constituent in cat milk quantitatively is fat, since it supplies most of the kitten's energy. The observation that fat concentration increases during lactation is similar to that found for the cow (17) and the dog (11), but is in contrast to reports for human (27) and rat milk (2) in which fat concentrations are not significantly influenced by lactation stage.

In conclusion, the composition of cats' milk was found to vary considerably with the stage of lactation. The concentrations of iron, copper, manganese, calcium, protein, carbohydrate and fat showed patterns that were influenced by the stage of lactation, whereas concentrations of zinc and magnesium, were not significantly affected. These data show that the nutrient intake of the suckling kitten changes markedly during the early neonatal period. Such changes should be taken into account when evaluating the trace element status of the growing kitten. Furthermore, lactational patterns should be considered when evaluating the cat as a model for trace element nutrition in humans with specific attention given to similarities and differences.

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