Changes in protein and nutrient composition of milk throughout lactation in dogs

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Objective—To evaluate changes in protein and nutrient composition of milk throughout lactation in dogs.

Sample Population—Milk samples collected from 10 lactating Beagles.

Procedure—Milk samples were collected on days 1, 3, 7, 14, 21, 28, 35, and 42 after parturition and analyzed to determine concentrations of nitrogen, nonprotein nitrogen, casein, whey proteins, amino acids, lipids, lactose, citrate, minerals, and trace elements. Optimum conditions for separating casein from whey proteins and distribution of milk proteins throughout lactation were assessed by use of polyacrylamide gel electrophoresis.

Results—Protein concentration was high in samples collected on day 1 (143 g/L), decreased through day 21 (68.4 g/L), and increased thereafter. Concentration of nonprotein nitrogen did not change throughout lactation (5.7 to 9.9% of total nitrogen content). Casein-to-whey ratio was approximately 70:30 and remained constant throughout lactation. Lactose concentration increased from 16.6 g/L on day 1 to 34.0 to 40.2 g/L on days 7 to 42. Lipid concentration ranged from 112.5 to 137.2 g/L. Citrate concentration increased from day 1 (4.8 mM) to day 7 (6.6 mM), then gradually decreased until day 42 (3.9 mM). Iron, zinc, copper, and magnesium concentrations decreased during lactation, whereas calcium and phosphorus concentrations increased. Calcium-to-phosphorus ratio remained constant throughout lactation (approx 1.6:1). Energy content of milk ranged from 1,444 to 1,831 kcal/L.

Conclusions and Clinical Relevance—Protein and nutrient composition of milk changes throughout lactation in dogs. These data can provide valuable information for use in establishing nutrient requirements of puppies during the suckling period. (Am J Vet Res 2001;62:1266–1272)

It is widely accepted that milk from a lactating dam offers optimal nutritional support for a suckling neonate. However, when a dam cannot meet nutritional requirements of the puppies in her litter, such as in situations of a decreased milk supply, failure of onset of lactation, or a litter of puppies orphaned as a result of death of the dam, homemade and commercially available milk replacers serve as substitutes.1 Although the replacers should be formulated to reflect the nutrient composition of bitch’s milk, some formulations contain inadequate amounts of protein, fat, calcium, and phosphorus.2 On the other hand, some formulas designed to be fed to puppies are fortified with nutrients in attempts to achieve a faster growth rate.3 Such fortification of existing formulas has led to overnutrition in puppies and caused gastrointestinal problems as a result of high lactose4 and fat content.

Other researchers have investigated the composition of bitch’s milk2,5-7; however, reported values vary considerably, possibly as a result of differences in methods used, breed of dogs, stage of lactation, and whether samples were obtained, or duration of milking during sample collection. Nutrient composition of milk in several species depends on stage of lactation.2,4 In a limited number of studies, investigators have examined changes in composition of milk throughout lactation in dogs, but they have considered only a few time points during lactation2,4 or analyzed a limited number of nutrients.3 In the study reported here, protein and nutrient composition of milk from Beagles was determined longitudinally throughout lactation. Detailed information obtained from this study will help investigators establish nutrient requirements of puppies, and it will also aid in the formulation of milk replacers for puppies that more accurately resemble bitch’s milk.

Materials and Methods

Animals—Ten healthy lactating Beagles were used in the study. The bitches were allowed ad libitum access to a diet that met or exceeded the nutrient profiles for growth and reproduction established by the Association of American Feed Control Officials. On an as-fed basis, the diet contained the following nutrients: carbohydrate (32.0%); protein (32.0%); fat (21.0%); moisture (7.75%); ash (5.5%); and fiber (1.75%). Growth rates of puppies were monitored and considered normal, and all puppies were healthy. Each bitch nursed a mean of 6.9 puppies that were weaned at 7 weeks of age.

Collection of samples—A sample of colostrum was collected on the day of parturition (day 1), and milk samples were collected on days 3, 7, 14, 21, 28, 35, and 42 after parturition. Milk or colostrum was obtained by manual expression of the glands after IM administration of 4 units of oxytocin. Each sample typically was > 3 ml. All samples were frozen at −20 C until analysis.

Separation of casein and whey proteins from whole milk—To investigate the distribution of casein and whey proteins of milk throughout lactation, these 2 classes of milk proteins were separated. Acid precipitation of whole milk was accomplished by addition of 1 M HCl to achieve pH 4.0, 4.3, and 4.6. Samples then were ultracentrifuged (242,000 × g at 4 C for 1 hour), and fat, whey, and the casein pellet were removed. The casein pellet was lyophilized, and the whey fraction was frozen at −20 C until analysis. Determination of the optimal separation method was performed by use of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). The whey fraction also was analyzed for nitrogen content to enable us to calculate the casein-to-whey ratio.
incubated for 10 minutes at 4°C. Citrate concentration was determined in 0.05 ml of whey. Protein was precipitated with 1 ml of 0.1 M potassium carbonate solution. The mixture then was extracted again from the aqueous phase. Approximately 5 g of milk were dried (wet ash), and the resulting product was mixed with 10 ml of concentrated nitric acid followed by 5 ml double-deionized water to achieve a final volume of 5 ml. Samples were dried (wet ash) to a final volume of approximately 1 ml, placed in volumetric flasks, and then diluted with double-deionized water to achieve a final volume of 5 ml.

Concentrations of iron, copper, and zinc were determined by atomic absorption spectrophotometry, using an acetylene flame. To determine calcium and magnesium concentrations, 0.5 ml of milk were dried (wet ash), and the resulting product was mixed with 10 ml of concentrated nitric acid followed by 5 ml of concentrated perchloric acid. Digested samples were diluted with water to a final volume of 100 ml in volumetric flasks and further diluted with 2.5% lanthanum oxide and then analyzed by atomic absorption spectrophotometry, using a nitrous oxide flame. Phosphorus concentration in milk was determined on a flame photometer, following the procedure of absorbance at 450 nm.4

Mineral content—To analyze iron, copper, and zinc concentrations, milk samples (0.5 ml) were incubated overnight at room temperature (23°C) with 5 ml of concentrated nitric acid. Samples were dried (wet ash) to a final volume of approximately 1 ml, placed in volumetric flasks, and then diluted with double-deionized water to achieve a final volume of 5 ml. Concentrations of iron, copper, and zinc were determined by atomic absorption spectroscopy, using an acetylene flame. To determine calcium and magnesium concentrations, 0.5 ml of milk were dried (wet ash), and the resulting product was mixed with 10 ml of concentrated nitric acid followed by 5 ml of concentrated perchloric acid. Digested samples were diluted with water to a final volume of 100 ml in volumetric flasks and further diluted with 2.5% lanthanum oxide and then analyzed by atomic absorption spectrophotometry, using a nitrous oxide flame. Phosphorus concentration in milk was determined on a flame photometer, following the procedure of absorbance at 450 nm.4

Total lipid content—Amount of total lipid was determined by use of a modification of the Folch assay.4 Lipids were extracted by the addition of methanol (3 ml), methylene chloride (6 ml), and 0.7% sodium chloride (6 ml) to 1 ml of milk. After the mixture was centrifuged at 1,000 X g for 10 minutes, the methylene chloride fraction was removed, and lipids were extracted again from the aqueous phase. Approximately 5 g of anhydrous sodium sulfate then was added to the combined methylene chloride fractions and centrifuged at 1,000 X g for 10 minutes. The supernatant was decanted into an aluminum pan, evaporated at room temperature (23°C), and weighed to determine the amount of total lipid.

Energy content—Energy density of milk was calculated by use of the Atwater factors (physiologic fuel values).5

Table 1—Concentrations (mean ± SEM) of nutrients throughout lactation in milk of 10 healthy dogs

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Day of lactation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>14</td>
</tr>
<tr>
<td>True protein (g/L)</td>
<td>142.0 ± 19.2</td>
</tr>
<tr>
<td>NPN (g/L)</td>
<td>1.19 ± 0.05</td>
</tr>
<tr>
<td>NPN (% N)</td>
<td>5.7 ± 0.7</td>
</tr>
<tr>
<td>Casein (% total protein)</td>
<td>60.7 ± 4.1</td>
</tr>
<tr>
<td>Whey (% total protein)</td>
<td>39.3 ± 4.1</td>
</tr>
<tr>
<td>Lipid (g/L)</td>
<td>132.2 ± 16.7</td>
</tr>
<tr>
<td>Lactose (g/L)</td>
<td>16.3 ± 1.3</td>
</tr>
<tr>
<td>Citrate (mM)</td>
<td>4.3 ± 0.04</td>
</tr>
<tr>
<td>Iron (mg/L)</td>
<td>3.7 ± 0.29</td>
</tr>
<tr>
<td>Copper (mg/L)</td>
<td>1.3 ± 0.06</td>
</tr>
<tr>
<td>Zinc (mg/L)</td>
<td>5.0 ± 1.3</td>
</tr>
<tr>
<td>Magnesium (mg/L)</td>
<td>128.5 ± 17.8</td>
</tr>
<tr>
<td>Calcium (mg/L)</td>
<td>1363 ± 108</td>
</tr>
<tr>
<td>Phosphorus (mg/L)</td>
<td>935 ± 83</td>
</tr>
<tr>
<td>Ca:P</td>
<td>1.5</td>
</tr>
<tr>
<td>Energy (kcal/L)</td>
<td>1831 ± 506</td>
</tr>
</tbody>
</table>

*Day 1 = First day of lactation. 1Protein content was calculated by subtracting nonprotein nitrogen (NPN) content from total nitrogen content and multiplying the resulting difference by 6.25.
****Within a row, values with different superscript letters differ significantly (P < 0.05). ND = Not determined. Ca:P = Calcium-to-phosphorus ratio.
Amino acid content—Samples were analyzed by personnel in the Molecular Structure Facility at the University of California, Davis, using an automated analyzer. Milk samples were hydrolyzed under argon gas by the addition of 6 N HCl and incubated for 24 hours at 100°C. Samples were rotary vaporized and resuspended in lithium diluting buffer. Tryptophan concentration was determined, using methods described by Penke et al. Methionine and cysteine concentrations were determined, using methods described elsewhere.

Statistical analysis—A repeated-measures ANOVA with 1 within factor (time) and no grouping factor was performed on each variable. Mean ± SEM was calculated for all values at each time point. Means were compared, using the Tukey studentized range test. All analyses were performed by use of a computerized statistical program. Values of P ≤ 0.05 were considered significant.

Results

Protein and NPN content—Protein concentration was high in colostrum (143 g/L), decreased significantly by day 21, and then slightly increased throughout the duration of lactation (Table 1). The NPN fraction of milk represented 5.7 to 9.9% of total nitrogen content and did not change during lactation. The casein-to-whey protein ratio remained relatively constant (approx 70:30) throughout the course of lactation. The amount of casein as a proportion of total protein content was calculated as 60.7% on day 1, increased to 75.4% by day 3, and then decreased slightly through day 42. A significant decrease was observed for the amount of whey proteins as a proportion of total protein content between days 1 (39.3%) and 3
(24.6%); however, it then increased to a mean of 31.6% on day 42.

Protein composition—Results of SDS-PAGE revealed that adjusting the milk pH to 4.0, 4.3, and 4.6 was effective for separating caseins and whey proteins (Fig 1). Distribution of casein subunits appeared to remain constant throughout lactation (Fig 2). Two major casein subunits with molecular weights of approximately 29 and 33 kd were detected. In contrast, certain whey proteins increased or decreased throughout lactation (Fig 3). The major whey proteins had molecular weights of 14 to 15, 20 to 22, 60, and 75 kd, respectively.

Total lipid and lactose content—Although amounts did not differ significantly, mean total lipid content in milk was approximately 130 mg/L during the first 7 days, decreased to 112.5 mg/L, and then increased to 133.7 mg/L (Table 1). A significant effect of time on the concentration of lactose throughout lactation was revealed by results of the repeated-measures ANOVA. Mean lactose concentration was significantly lower in colostrum (16.6 g/L) than in milk obtained on subsequent days, but it increased to 40.2 g/L by day 28 and then decreased to a concentration similar to that of transitional milk (approx 35 g/L) throughout the remainder of lactation.

Energy content—Mean value for total energy content of milk decreased from days 1 (1,800 kcal/L) to 14 (1,400 kcal/L). Subsequently, it increased to 1,700 kcal/L by day 42 (Table 1).

Citrate content—A significant difference in citrate concentration was detected during lactation (Table 1). Mean citrate concentration increased from the sample of colostrum on day 1 (4.8 mM) to the milk sample obtained on day 7 (6.6 mM). It then gradually decreased to achieve a concentration similar to that of colostrum for samples obtained on days 28 (5.0 mM) and 33 (4.4 mM).

Trace element content—A significant effect of time was observed for iron concentrations in milk. Mean iron concentration increased significantly from days 1 (3.7 mg/L) to 3 (6.9 mg/L). It then gradually decreased to 1.8 mg/L by day 42 (Table 1). In contrast, mean zinc concentration in milk slowly increased from days 1 (5.0 mg/L) to 14 (6.1 mg/L), and it then decreased to 4.1 mg/L by day 42. However, significant differences were not detected among days of lactation. Mean copper concentration was slightly higher in early lactation (1.3 mg/L on day 1) and then gradually decreased to approximately 1 mg/L throughout lactation.

Mineral content—Mean calcium concentration in milk was lowest in colostrum (1,363 mg/L), but it increased significantly to 1,950 mg/L on day 14 and peaked at 2,440 mg/L on day 35. Mean magnesium concentration in milk was highest in colostrum (128.5 mg/L) but rapidly decreased to 85.8 mg/L on day 3 and remained relatively constant throughout lactation (Table 1). Mean concentration of phosphorus increased slightly over time (914 mg/L on day 3 to 1,401 mg/L on day 28), but these values did not differ significantly. The calcium-to-phosphorus ratio remained relatively constant in early lactation (approx 1.5:1) but increased to approximately 1.7:1 by late lactation.

Amino acid content—Results of repeated-measures ANOVA revealed a significant effect of time on the concentrations for all amino acids in milk, except tryptophan (Table 2). For all amino acids, the largest difference was detected between days 1 and 3. Tryptophan was not detected in some milk samples; thus, SEM could be calculated only for days 1, 35, and 42.
Table 2—Amino acid composition (mean ± SEM) millimole per liter throughout lactation in milk of 10 healthy dogs

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>0.170±0.012</td>
<td>0.052±0.007</td>
<td>0.082±0.010</td>
<td>0.082±0.008</td>
<td>0.082±0.009</td>
<td>0.076±0.010</td>
<td>0.096±0.014</td>
<td>0.094±0.010</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.104±0.007</td>
<td>0.032±0.004</td>
<td>0.056±0.008</td>
<td>0.059±0.009</td>
<td>0.061±0.007</td>
<td>0.052±0.010</td>
<td>0.144±0.032</td>
<td>0.142±0.009</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.235±0.015</td>
<td>0.073±0.009</td>
<td>0.117±0.014</td>
<td>0.120±0.011</td>
<td>0.120±0.025</td>
<td>0.113±0.015</td>
<td>0.069±0.008</td>
<td>0.066±0.009</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.058±0.004</td>
<td>0.021±0.003</td>
<td>0.032±0.005</td>
<td>0.031±0.003</td>
<td>0.029±0.003</td>
<td>0.027±0.004</td>
<td>0.030±0.003</td>
<td>0.031±0.002</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.426±0.021</td>
<td>0.124±0.018</td>
<td>0.242±0.004</td>
<td>0.243±0.032</td>
<td>0.245±0.023</td>
<td>0.141±0.034</td>
<td>0.277±0.032</td>
<td>0.270±0.022</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.076±0.004</td>
<td>0.021±0.003</td>
<td>0.041±0.007</td>
<td>0.032±0.010</td>
<td>0.042±0.005</td>
<td>0.036±0.008</td>
<td>0.037±0.011</td>
<td>0.038±0.010</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.128±0.007</td>
<td>0.037±0.005</td>
<td>0.069±0.011</td>
<td>0.079±0.009</td>
<td>0.072±0.008</td>
<td>0.062±0.012</td>
<td>0.063±0.010</td>
<td>0.060±0.008</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.301±0.017</td>
<td>0.093±0.014</td>
<td>0.169±0.026</td>
<td>0.169±0.024</td>
<td>0.173±0.019</td>
<td>0.147±0.030</td>
<td>0.195±0.022</td>
<td>0.191±0.019</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.128±0.008</td>
<td>0.037±0.005</td>
<td>0.061±0.008</td>
<td>0.061±0.007</td>
<td>0.062±0.009</td>
<td>0.058±0.008</td>
<td>0.075±0.008</td>
<td>0.073±0.009</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.073±0.005</td>
<td>0.022±0.003</td>
<td>0.036±0.005</td>
<td>0.037±0.004</td>
<td>0.033±0.005</td>
<td>0.042±0.005</td>
<td>0.042±0.002</td>
<td>0.042±0.008</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.096±0.006</td>
<td>0.027±0.004</td>
<td>0.048±0.007</td>
<td>0.048±0.006</td>
<td>0.049±0.009</td>
<td>0.044±0.008</td>
<td>0.058±0.006</td>
<td>0.057±0.005</td>
</tr>
<tr>
<td>Proline</td>
<td>0.302±0.017</td>
<td>0.083±0.013</td>
<td>0.168±0.028</td>
<td>0.165±0.026</td>
<td>0.171±0.020</td>
<td>0.141±0.034</td>
<td>0.189±0.023</td>
<td>0.178±0.016</td>
</tr>
<tr>
<td>Serine</td>
<td>0.128±0.007</td>
<td>0.036±0.005</td>
<td>0.065±0.009</td>
<td>0.064±0.009</td>
<td>0.064±0.009</td>
<td>0.064±0.007</td>
<td>0.216±0.048</td>
<td>0.220±0.046</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.149±0.009</td>
<td>0.045±0.006</td>
<td>0.070±0.007</td>
<td>0.070±0.005</td>
<td>0.068±0.015</td>
<td>0.061±0.008</td>
<td>0.075±0.007</td>
<td>0.076±0.004</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.077±0.002</td>
<td>ND</td>
<td>0.005</td>
<td>0.004</td>
<td>0.003</td>
<td>0.003</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.068±0.005</td>
<td>0.031±0.005</td>
<td>0.056±0.004</td>
<td>0.034±0.004</td>
<td>0.035±0.004</td>
<td>0.031±0.006</td>
<td>0.041±0.005</td>
<td>0.041±0.003</td>
</tr>
<tr>
<td>Valine</td>
<td>0.206±0.013</td>
<td>0.060±0.008</td>
<td>0.102±0.014</td>
<td>0.100±0.011</td>
<td>0.101±0.010</td>
<td>0.088±0.015</td>
<td>0.113±0.012</td>
<td>0.111±0.008</td>
</tr>
</tbody>
</table>

*Day of lactation:
1: 1 to 4 days postpartum
3: 5 to 7 days postpartum
7: 8 to 10 days postpartum
14: 11 to 13 days postpartum
21: 14 to 20 days postpartum
28: 21 to 27 days postpartum
35: 28 to 34 days postpartum
42: 35 to 41 days postpartum

1For all amino acids, values differed significantly (P < 0.001) over time, except values for tryptophan (P = 0.488).
2Within a row, values with different superscript letters differ significantly (P < 0.05).
See Table 1 for remainder of key.

Discussion

In the study reported here, protein concentration during the first 7 days of lactation was approximately 100 g/L, whereas it was only approximately 80 g/L in milk during subsequent days of lactation. These concentrations are higher than those reported elsewhere,12,13 which is possibly a reflection of differences in analytic methods. Protein concentrations as low as 40 g/L in early lactation and up to 70 g/L in milk obtained later during lactation have been reported.12,13 Similar to milk from humans and cows, we observed a gradual pattern of decreasing protein concentrations with increasing duration of lactation. In contrast, other investigators found an increase in protein concentration as lactation progressed12 or a protein concentration that remained fairly constant throughout lactation.11 Concentration of NPN in milk from dogs was approximately 2.5-fold higher than values reported for most other species,12,22,24 but it was similar to concentrations in milk from cats.9 The NPN fraction represented 6 to 10% of total nitrogen content in milk from dogs, which is similar to the 7 to 9% found in milk from cats,4,3 to 5% in milk from cows,24 and 2 to 14% in milk from mares.23 However, it is considerably less than the 20 to 25% found in human milk.6

Unlike milk from humans and cats but similar to milk from cows, casein makes up most of the total protein content in milk from dogs during early lactation. The change from a predominance of whey to a predominance of casein throughout lactation in milk from humans, horses, and cats13,15,16 was not detected in milk from dogs. Also, the casein-to-whey ratio remained relatively constant (approx 70:30) throughout lactation, which may have been correlated with the lack of significant change in the concentration of amino acids throughout lactation. To our knowledge, longitudinal changes in casein-to-whey ratio and amino acid concentrations in milk from dogs have not been reported elsewhere.

We documented that decreasing the pH of milk from dogs to between 4.0 and 4.6 followed by ultracentrifugation effectively separated caseins from whey proteins. This method also would be useful in the initial purification process of specific proteins in milk of dogs. Although we did not identify the various casein subunits, SDS-PAGE revealed a 29-kd protein and a 33-kd protein that probably were β-casein and κ-casein, respectively. Casein in milk has a role in mineral absorption.7 It has been reported that casein in human milk binds a substantial fraction of magnesium, iron, copper, and zinc.20 Thus, it is possible that the high amount of casein in milk from dogs may bind a considerable amount of minerals and trace elements, enhancing mineral bioavailability in newborn puppies by efficiently delivering the minerals to absorption sites in the intestines. In addition, it has been suggested that casein has opiate-like effects29 and antiadhesive properties that prevent the attachment of bacteria to the mucosal lining by acting as a receptor analogue.20 Whether caseins in milk from dogs exert similar functions has not been investigated.

Similar to the situation in many other species, it is possible that milk of dogs contains proteins that have physiologic functions that actively contribute to the development of puppies. For example, milk of dogs contains lysozyme, a bacteriolytic enzyme that prevents the growth of several bacteria,31 and bile salt-stimulated lipase, which aids in lipid digestion.32 Identification of such bioactive proteins in milk of dogs suggests that these proteins interact with other nutrients in bitch’s milk and facilitate their utilization by puppies. Although the study reported here did not conclusively identify the major whey proteins, molecular weights from SDS-PAGE suggest that the major pro-
proteins would correspond to α-lactalbumin (14 kd), β-lactoglobulin (15 kd), β-lactoglobulin variants (20 to 22 kd), serum albumin (68 kd), and secretory IgA sub-units (15, 30, 55, and 75 kd). Similar to the situation for caseins in milk of dogs, additional studies are needed to elucidate the roles these whey proteins play in puppies.

Overall, concentrations of amino acids in milk proteins from dogs were similar to those in humans.8 We detected a pattern of decreasing concentrations for all amino acids with increasing lactational stage, which is comparable to that for milk from humans.9

In our study, concentration of lactose in milk of dogs was similar to that reported by other investigators.4,12,13 In other species, lactose concentration in milk correlates negatively with protein content as lactation progresses.9,13,35 Analysis of our findings revealed a similar pattern, because lactose concentration was lowest in colostrum and highest in milk obtained subsequently during lactation, whereas protein concentration decreased as lactation progressed.

Concentration of lipids in milk from dogs determined in the study reported here was comparable to values found in milk from cats4 but higher than in milk from cows2 and humans.36 Concentration of lipids in milk from dogs reported here was similar to that reported in other species.8,13,35; however, our concentrations were higher than in another study on composition of milk from dogs.35 This difference may be attributable to the method of lipid determination or to factors such as composition of maternal diet, milking techniques, or the duration of milking during sample collection, because milk collected later during milking has a higher fat content than fore milk.36-40 In our study, milking sessions lasted 10 to 20 minutes, which may have provided sufficient time to obtain a representative milk sample.

Total energy content calculated here was higher in milk from dogs than in milk from cats,4 which is attributable to higher protein and lipid contents in milk from dogs. Interestingly, total energy in milk from dogs and cats decreases gradually from colostrum to milk obtained during mid-lactation, but it then increases again throughout the remainder of lactation.

Similar to citrate concentrations in milk from humans2 and cats,8 citrate concentrations in milk from dogs decreased throughout lactation. Citrate can chelate minerals such as iron, zinc, and magnesium and increase iron and zinc absorption from milk.13,35 It is possible that the observed decrease in iron and zinc concentrations in milk from dogs throughout lactation may be associated with a decrease in citrate concentration during the same period.

Although iron concentrations in this study (1.8 to 6.9 mg/L) were lower than values reported elsewhere (6 to 11 mg/L),13 the pattern of decreasing concentrations throughout the course of lactation was similar. Iron concentration was lower in colostrum (3.7 mg/L) than in samples obtained during days 3 to 14 (approx 6 mg/L), and it then decreased to > 2 mg/mL. In contrast in humans and cows, concentrations are high in colostrum and then gradually decrease during lactation.14 Dogs are born with large hepatic stores of iron,15 which may compensate for the low iron concentration in colostrum. It is possible that the mechanism for transport of iron into the mammary glands is not fully developed or that proteins involved in binding of trace elements are not fully synthesized or functional in early lactation. Concentration of copper in milk from dogs reported here was similar to that in other studies on milk from dogs2,12 and cats8 (approx 1 mg/L), but it was substantially higher than concentrations found in milk from humans2 and cows (approx 0.2 mg/L).15 The highest concentration was detected during early lactation, and it decreased throughout lactation, with the largest change during the first 14 days. This pattern has been reported for milk from humans.16 Similar to other species,26 it is possible that a high percentage of copper in milk from dogs is bound to caseins as well as to low-molecular weight ligands such as citrate and free amino acids. In our study, zinc concentration in milk from dogs (approx 5 mg/L) was slightly less than values reported elsewhere (7 to 8 mg/L)12; however, concentrations were higher than those reported for milk from humans during mid to late lactation8 (1 to 3 mg/L) and for milk from cows (3 mg/L).15 It is possible that the higher zinc and iron concentrations reported in other studies may be attributable to contamination of the teats with blood.

A marginal effect of time was detected for magnesium concentration in milk from dogs. The largest change was detected between days 1 and 3; it then achieved a plateau throughout the duration of lactation. Our values (approx 100 mg/L) are slightly higher than those reported in another study (60 mg/L).15 Calcium concentrations in milk from dogs increased markedly from days 1 through 35, reaching a value of 2,440 mg/L. These values are comparable to concentrations reported in other studies on milk from dogs.7,12 Also, calcium concentrations in milk obtained during mid to late lactation were similar to values for milk from other carnivores such as cats,8 but they were 5- to 6-fold higher than those in milk from horses, ruminants, and humans.20,41-46 At all time points during lactation, phosphorus concentrations were lower than calcium concentrations, yielding a calcium-to-phosphorus ratio of approximately 1.5:1. This ratio is similar to that reported in other species.4,60

We evaluated the protein and nutrient composition of milk from dogs to provide more information for use in establishing nutrient requirements of puppies. To our knowledge, this is the first report of nitrogen distribution, protein and amino acid patterns, and citrate and phosphorus concentrations in milk from dogs. Although we cannot exclude the possibility that the nutrient composition of milk (eg, energy content) may differ among breeds of dogs and that the diet of a bitch may influence some nutrients in her milk, this study provides an in-depth database of longitudinal changes in milk composition during lactation of healthy Beagles fed a typical diet formulated for dogs.

Precaust 10 to 18% gradient gel, Owl Scientific, Portsmouth, NH.
Brilliant Blue R, Sigma Chemical Co, St Louis, Mo.
Kjeltec auto 1030 analyzer, Tecator, Höganäs, Sweden.
Citric acid kit, Roche Molecular Biochemicals, Indianapolis, Ind.

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References


