Comparison of the nutrient composition of commercial dog milk replacers with that of dog milk

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Objective—To compare the nutrient composition of commercially available dog milk replacers with that of dog milk.

Design—Prospective, cross-sectional study.

Sample—5 dog milk samples and 15 samples of commercial dog milk replacers.

Procedures—Dog milk and milk replacers were analyzed for concentrations of total protein, essential amino acids, sugars, total fat, essential fatty acids, calcium, and phosphorus. Energy density was calculated. Results from milk replacers were compared with the range of the concentration of each nutrient in milk samples from mature dogs as well as the National Research Council (NRC) recommendations for puppy growth.

Results—Milk replacers varied widely in caloric density and concentration of nutrients such as calcium, protein, and fat. Calcium concentration was lower in 14 of 15 milk replacers than in the dog milk samples. Docosahexaenoic acid was undetectable in 12 of 15 milk replacers but present in all dog milk samples. All milk replacers had numerous essential nutrients outside of the range of the dog milk samples, and many had concentrations of amino acids, essential fatty acids, calcium, and phosphorus less than the NRC minimal requirement or recommended allowance. Compared with NRC recommendations, some dog milk samples had concentrations of total protein, linoleic acid, calcium, or phosphorus less than the recommended allowance.

Conclusions and Clinical Relevance—Results suggested that there was substantial variation in nutrient composition of 15 dog milk replacers and that some products were closer approximations of dog milk than others. Nearly all products would benefit from more appropriate calcium, amino acids, and essential fatty acids concentrations and better feeding directions. (*J Am Vet Med Assoc* 2014;244:1413–1422)

Commercial milk replacers for puppies have been available for decades and are commonly used for rearing orphans, for ill or weakened neonates, to supplement dog milk for large litters, and to mix with commercial puppy diets during the weaning process. Numerous products are available, but unlike other pet foods, the nutritional adequacy standards these products are expected to meet are not clearly defined. These factors make it difficult for pet owners and veterinarians to select appropriate milk replacers for their needs. Indeed, there is evidence that dog milk replacers may be quite variable in essential macronutrient and mineral concentrations among specific products and in comparison to dog milk.[1–3] However, the authors are unaware of any recent studies investigating the essential nutrient composition of a wide range of milk replacer products available in the United States, compared with contemporaneously analyzed dog milk, and this information is critical to allow educated decisions to be made on product use.

Abbreviations

ALA	α-Linolenic acid
ARA	Arachidonic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
MR	Minimal requirement
NRC	National Research Council
PUFA	Polyunsaturated fatty acids
RA	Recommended allowance

Fatty acid composition of milk replacers is of particular interest because PUFA of the n-6 and n-3 families are increasingly recognized as critical for proper neonatal development.[4] In particular, ARA (20:4 n-6) and DHA (22:6 n-3) are the 2 main PUFA in the brain. These fatty acids are critical for normal retinal and neural development in mammals[5–7] and have been supplemented in human infant formulas since 2002.[8] The difference in fatty acid composition between dog milk replacers and milk from domestic dogs remains an unexplored area of research; however, there is evidence in

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other species to suggest that milk replacers are often lower in long-chain PUFA, compared with milk. Prior to DHA and ARA supplementation of human infant formula, these products contained low concentrations of DHA and ARA, compared with human milk.[9] Two studies[10,11] have investigated the fatty acid composition of 4 cat milk replacers, compared with that of domestic cat milk. Both studies[10,11] found that the milk replacers contained no detectable concentrations of DHA. Arachidonic acid concentrations ranged from undetectable to 60% of the concentration in cat milk in both studies; 3 of 4 products contained concentrations < 16% of the concentration in cat milk.

The purpose of the study reported here was to compare the concentrations of various nutrients in commercially available dog milk replacers with those in dog milk. We hypothesized that the commercial dog milk replacers would differ from dog milk in macro- and micronutrient profiles and would be lower in concentrations of long-chain PUFA (especially ARA and DHA), compared with dog milk.

Materials and Methods

Commercial milk replacers—Fifteen commercially available products marketed as milk replacers (7 liquid and 8 powdered)[a–o] for newborn puppies were purchased from local retail stores and online sellers. If both liquid and powered versions were available of the same brand, the liquid version was selected for convenience and cost savings to maximize the number of products that could be analyzed. All expiration dates were checked prior to sampling to ensure that no product was expired or would expire within 1 month.

All powdered versions were reconstituted with deionized water according to the manufacturers' directions, with the exception of the samples used for amino acid analysis, which were submitted as powders directly from the original packaging. The amount of powder used for reconstitution was weighed in grams and recorded for each powdered product that was reconstituted. All liquid products were sampled directly from the container immediately after opening. Each product was assigned a code number and then aliquoted into multiple smaller volumes for storage and analysis. All aliquots were stored at –80°C until analysis.

Dog milk collection—Local owners of female breeding dogs were invited to donate dog milk for the study. The collection protocol was approved by the Cummings School of Veterinary Medicine at Tufts University Clinical Studies Review Committee prior to the start of the study. Breeders were identified through local breed clubs, dog organizations, and veterinarians. Inclusion criteria for dogs included a body weight > 13.64 kg (30 lb) to help ensure adequate milk volume could be collected, between 2 and 3 weeks after parturition at the start of collection, and no known medical problems. Additionally, all dogs were consuming diets appropriate for gestation and lactation as determined on the basis of the product nutritional adequacy statements and were not supplemented with nutraceuticals or additional sources of vitamins, minerals, or herbs. A standardized diet and health history was collected for each dog prior to enrollment in the study to ensure that all inclusion criteria were met.

Exclusion criteria included dogs fed home-prepared diets, those fed diets high in n-3 fatty acids, and those receiving extra fatty acids or fatty acid–containing meats (ie, fish, flax, or vegetable oils; salmon; or fish) in addition to a standard commercial diet. Diets were considered to be high in n-3 fatty acids if they contained 1.5 g/1,000 kcal of total n-3 fatty acids, per the manufacturer.

Milk was manually expressed by the owner into sterile conical tubes daily (1 tube for each day) for 3 days, at various times of the day and from a variety of teats, with the goal of a total volume of 5 to 8 mL of milk/d. Each day's sample was kept capped and refrigerated between and after collection. Within 24 hours after collection of the last day's sample, all samples were collected by the investigators and transported on ice to the laboratory. Samples were then warmed to dog body temperature (39°C [102.2°F]) in a heat block and inverted repeatedly to resuspend any cream layer that had separated. Samples for the 3 days were then pooled and aliquoted into smaller samples before being immediately frozen at –80°C. Amino acid profiles, fatty acid composition, proximate analysis, calcium, phosphorus, and sugars were measured for all milk replacer and dog milk samples.

Proximate analyses and energy determination—Milk and milk replacer samples were analyzed in accordance with standard methods.[12,p] All constituents were analyzed in duplicate, with results reported as g/1,000 kcal on the basis of the calculated gross energy. Gross energy was calculated assuming 9.11 kcal/g for fat, 5.86 kcal/g for protein, and 3.95 kcal/g for sugar and was expressed as kilocalories per gram of milk.[13] This calculation may overestimate gross energy because it does not account for nonprotein nitrogen[14]; however, gross energy values calculated by this formula were not different from gross energy values determined by bomb calorimetry for rhesus macaque milk.[15] The gross energy value was then converted from kilocalories per gram to kilocalories per milliter, on the basis of the weight of 1 mL of each sample (all samples were individually weighed). These values were then used to convert data from the percentage of the nutrient as-fed to g/1,000 kcal gross energy.

For dry matter determination, milk and milk replacer samples were aliquoted, weighed, and dried in a forced-air drying oven for 3 hours at 100°C and then reweighed.[16] Total nitrogen was determined for these dried samples with a carbon, hydrogen, and nitrogen elemental gas analyzer[q] at a combustion temperature of 950°C, with supplemental oxygen boosts of 2 seconds to ensure complete combustion. This method has been validated against the macro Kjeldahl procedure with nitrogen recovery of approximately 98% to 99% and has been used at this laboratory to measure milk nitrogen for a wide variety of species.[17] The total nitrogen value was multiplied by 6.38 to determine the amount of crude protein in the milk.[18] Total lipid was measured in a microscale modification of the Roese-Gottlieb procedure involving sequential extractions with diethyl ether and petroleum ether after disruption of milk fat

globules with ammonium hydroxide and ethanol.[17] Total carbohydrate was analyzed with the phenol-sulfuric acid colorimetric procedure,[19,20] with lactose monohydrate used to prepare standards; absorbance was read at 490 nm with a UV-visible spectrophotometer[r] equipped with an automatic sipper tube.

Minerals—For total mineral (ash) estimates, milk samples were dried in crucibles and combusted in a muffle furnace at 550°C for 8 hours. For specific mineral analyses, the ash from the procedure was digested in nitric and perchloric acid on a hot plate contained in a perchloric-acid rated fume hood. Mineral digests were diluted in deionized water and analyzed for calcium via flame atomic absorption spectrophotometry[s] at 422.7 nm. Phosphorus was analyzed by use of the Association of Official Analytical Chemists modified Gomori method[17] and read with a microplate reader[t] at 450 nm.

Fatty acid analysis—Fatty acids from the dog milk and milk replacer formula preparations were isolated and methylated by means of a modification of the method described by Folch et al.[21] Briefly, 100 µL of either milk or milk replacer was added to 0.4 mL of PBS solution. After the addition of internal standard (30 µg of heptadecanoic acid), the samples were mixed with 3.0 mL of a mixture of chloroform and methanol (2:1 vol/vol) and vortexed. After incubation on ice for 10 minutes, the samples were vortexed and centrifuged at 1,080 × *g* for 10 minutes. The bottom infranatant was removed and completely dried under nitrogen gas vapors. The dried samples were then methylated by the addition of 0.5 mL of methanolic NaOH and incubated for 3 minutes at 100°C.

Samples were allowed to cool, 0.5 mL of boron trifluoride in methanol was added, and the samples were incubated at 100°C for 1 minute. After cooling, the samples were mixed with 1.0 mL of hexane, followed by 6.5 mL of a saturated saline (0.9% NaCl) solution to help separate the methyl esters from the rest of the solution. The samples were then vortexed and centrifuged at 500 × *g* for 4 minutes, and the upper hexane phase was transferred to a fresh vial and quantified by gas chromatography–mass spectroscopy.[u,v] From these data, n-6:n-3 ratios were calculated as (linoleic acid + ARA)/(ALA + DHA + EPA).

Amino acid analysis—Dog milk and liquid milk replacer samples were dried in protein hydrolysis ampoules prior to analysis with a concentrator.[w] This step was not used for powdered milk replacers because they were already in an appropriate form. For determination of all amino acids except cystine, methionine, and tryptophan, dried milk protein was hydrolyzed under nitrogen gas by the addition of 6N HCl and was incubated for 24 hours at 110°C.[22] Cystine and methionine were determined in accordance with the performic acid oxidation with acid hydrolysis and hydrobromic acid method.[22] Analysis for tryptophan was completed by means of a described standard method.[23] All samples were filtered with a 0.45-mm filter[x] and analyzed with an automated amino acid analyzer[y] by means of cation-exchange high-performance liquid chromatography and ninhydrin-reactive colorimetric detection.

Sugar analysis—All samples were assayed for glucose, fructose, and lactose via ion chromatography at a commercial laboratory.[z] The lower limit of quantification was 50 µg/g (50 mg/kg) and lower values were assigned to be 0 to facilitate statistical analysis.

Statistical analysis—The investigators and all laboratory personnel were unaware of the identity of the various milk replacer and dog milk samples until data analysis was complete. All data were converted into comparable units (g/1,000 kcal gross energy) for comparison of milk replacers with dog milk. For comparison with the NRC recommendations, all data were converted to g/1,000 kcal metabolizable energy with standard human Atwater factors. All data were then evaluated graphically and on the basis of an Anderson-Darling test for normality. Data from the milk samples were generally normally distributed; however, most of the data for the milk replacers were skewed. Descriptive statistics were calculated for the milk samples and milk replacer samples with the aid of a commercial statistical program.[aa] Nutrient concentrations and caloric density of the individual formula samples were compared with the ranges of nutrient concentration for all 5 milk samples. Both milk and formula data were compared with the NRC MR and RA for growing puppies 4 to 14 weeks of age (total protein and amino acids) or growing puppies after weaning (other nutrients).[24] Combined data from either dog milk or milk replacers are presented as median (range). All nutrients are presented on a g/1,000 kcal of gross energy or metabolizable energy basis, as appropriate, to allow comparison among products of different caloric density, given that each product would need to be fed to meet the puppies' caloric requirement rather than on a volume or weight basis.

Results

Milk was obtained from 5 healthy dogs with a median age of 4 years (range, 2 to 4 years). Breeds represented were Australian Shepherd Dog (n = 1), Boxer (1), Golden Retriever (2), and Newfoundland (1). The dogs were nursing a median of 6 puppies (range, 5 to 9 puppies), and the first of the 3 daily samples was collected a median of 15 days (range, 14 to 24 days) after parturition. Although the initial inclusion criterion was that dogs should be included between 2 and 3 weeks after parturition, there was some confusion over 1 whelping date, which led to inclusion of 1 dog at 24 days after parturition.

Dog milk samples—In 1 dog milk sample, protein concentration was below the NRC RA (47.4 vs 56.3 g/1,000 kcal metabolizable energy). All 5 milk samples exceeded both the NRC MR and RA for 4- to 14-week-old puppies for all amino acids except tryptophan, lysine, and threonine. The sample that contained less than the RA for protein concentration also did not meet the RA and contained slightly less than the MR (0.44 vs 0.45 g/1,000 kcal) of tryptophan. The same sample also contained slightly less than the RA of threonine (2.01 vs 2.03 g/1,000 kcal) and lysine (2.06 vs 2.20 g/1,000 kcal). Two of the 5 milk samples contained much less than the RA for linoleic acid (no MR is available). Two of the dog milk samples contained less than the NRC MR

for calcium, and 4 out of 5 samples contained less than the RA. Phosphorus concentration was much less than the RA for all milk samples (no MR is available). Descriptive data for dog milk samples were summarized (**Tables 1–4**).

Milk replacer products versus dog milk—Nutrient concentrations varied widely among the 15 milk replacer products sampled, and none of the 15 milk replacers were close matches for dog milk. For the 21 essential nutrients that were analyzed, none of the 15

Table 1—Caloric density (gross energy basis) of 15 dog milk replacer samples, compared with values for 5 dog milk samples.

	Dog milk samples		Milk replacer samples		
Variable	**Median**	**Range**	**Median**	**Range**	**Interquartile range**
Calories (kcal/kg)	1,477	1,180–1,622	940	477–1,416	688–1,059
Calories (kcal/mL)	1.56	1.2–1.62	0.96	0.49–1.49	0.76–1.11

Table 2—Values for protein and essential amino acid concentrations (g/1,000 kcal metabolizable energy) of 15 dog milk replacer samples, compared with 5 bitch milk samples and the NRC MR and RA for each nutrient.

			Dog milk samples		Milk replacer samples		
Nutrient	**NRC MR***	**NRC RA***	**Median**	**Range**	**Median**	**Range**	**Interquartile range**
Protein	45	56.3	56.7	43.6–74.0	58.3	50.4–101.2	53.5–62.3
Arginine	1.58	1.98	3.34	2.40–3.88	2.2	1.26–3.74	1.70–2.75
Histidine	0.78	0.98	1.70	1.40–1.99	1.32	0.91–2.05	1.18–1.54
Isoleucine	1.3	1.63	2.60	2.08–2.94	2.85	2.19–5.19	2.56–3.65
Leucine	2.58	3.22	6.99	5.59–8.17	5.58	4.06–9.14	4.59–7.36
Lysine	1.75	2.2	2.55	1.89–2.91	4.45	3.20–7.12	3.57–5.70
Methionine	0.7	0.88	1.54	1.11–1.79	1.50	0.84–3.37	1.25–2.03
Methionine and cystine	1.4	1.75	2.45	1.77–2.80	2.25	1.08–4.48	1.60–3.24
Phenylalanine	1.3	1.63	2.45	1.84–2.87	2.62	1.65–4.12	2.28–3.06
Phenylalanine and tyrosine	2.6	3.25	4.70	3.40–5.24	5.34	3.25–8.35	4.63–6.06
Threonine	1.63	2.03	2.52	1.85–2.89	2.81	1.91–6.15	2.30–4.00
Tryptophan	0.45	0.58	0.57	0.40–0.72	0.78	0.44–1.43	0.64–1.17
Valine	1.35	1.7	3.11	2.45–3.69	3.48	2.71–5.46	3.09–4.15

*The NRC values are for puppies ≥ 4 weeks of age eating solid foods; however, the dog milk was collected between 14 and 24 days after parturition.

Table 3—Total fat, essential fatty acid, and mineral content (g/1,000 kcal metabolizable energy) in 15 dog milk replacer samples, compared with values for 5 dog milk samples and the NRC RA for each nutrient.

		Dog milk samples		Milk replacer samples		
Nutrient	**RA**	**Median**	**Range**	**Median**	**Range**	**Interquartile range**
Total fat	21.3	62.5	54.8–72.8	56.4	28.4–72.0	48. 7–62.7
Linoleic acid	3.3	3.21	1.14–6.03	3.40	0.43–10.83	1.65–7.70
ALA	0.2	0.19	0.06–0.66	0.29	0.03–1.47	0.11–1.08
ARA	0.08	0.24	0.09–0.58	0.01	0–0.08	0.01–0.03
EPA	—	0.09	0.07–0.12	0.00	0–0.53	0.00–0.01
DHA	—	0.16	0.13–0.24	0.00	0.00–0.47	0.00–0.00
EPA and DHA	0.13	0.25	0.20–0.36	0.00	0.00–1.0	0.00–0.01
n-6:n-3 ratio	—	6.15	4.47–7.56	6.05	2.01–35.9	5.28–19.46
Calcium*	3	2.13	1.65–2.89	1.21	0.73–1.67	0.99–1.41
Phosphorus	2.5	1.37	0.94–1.61	1.13	0.65–1.95	0.91–1.23
Calcium-to-phosphorus ratio	1.2	1.72	1.20–3.06	1.02	0.62–1.70	0.92–1.33

*The NRC minimal requirement for calcium is 2 g/1,000 kcal metabolizable energy for puppies ≥ 4 weeks of age eating solid foods.
— = Not applicable.

Table 4—Sugar content (g/1,000 kcal gross energy) in 15 dog milk replacer samples, compared with values for 5 dog milk samples.

	Dog milk samples		Milk replacer samples		
Nutrient	**Median**	**Range**	**Median**	**Range**	**Interquartile range**
Total sugars	23.7	20.2–34.5	35.1	22.0–99.3	30.0–50.2
Glucose	0.05	0.04–0.08	0.70	0.10–6.87	0.67–1.24
Sucrose	0.07	0.00–1.27	0.00	0.00–4.36	0.00–0.15
Lactose	20.0	18.1–32.1	22.5	8.10–92.8	17.9–28.6
Fructose	0.00	0.00–0.04	0.06	0.00–0.76	0.00–0.15

milk replacers had all essential nutrients within the range of the dog milk samples. The number of essential nutrients (including energy density; n = 22) outside the range for dog milk ranged from 11 to 18, whereas the overall number of nutrients analyzed (including energy density; 30) outside the range for dog milk ranged from 15 to 24. Overall, even the 3 milk replacers[f,m,o] that were the closest matches to dog milk (on the basis of the total number of analyzed nutrients within the dog milk range) had potentially important nutritional issues, such as no measurable DHA,[f,m,o] excessive linoleic acid,[m] low energy density,[f,o] and inappropriate calcium-to-phosphorus ratios.[f]

Only 3 of 15 milk replacer products contained gross energy within the range of the 5 dog milk samples (Table 1). Despite a 3-fold difference in energy density among the milk replacers, 12 of the 15 products provided usage instructions that indicated that 2 tablespoons (30 mL of appropriately reconstituted milk replacer or, for 3 products, at least 2 tablespoons) should be fed to a 113-g (4-oz) puppy. Two milk replacer products did not provide any feeding directions, and another product, which was the third lowest in energy density (0.71 kcal/mL of gross energy), recommended 1.5 tablespoons (22.5 mL) for a 113-g puppy.

All 15 milk replacers had a total protein concentration within or higher than the range for dog milk (Table 2). However, all essential amino acid concentrations were within or greater than the range of dog milk for only 3 of the 15 milk replacer samples. Histidine (9/15 milk replacer samples), arginine (8/15), and leucine (8/15) concentrations were frequently less than the dog milk range. Despite this, almost all milk replacers exceeded the NRC RA for 4- to 14-week-old puppies for histidine (14/15) and leucine (15/15). For arginine, 1 milk replacer had less than the NRC MR (1.38 vs 1.58 g/1,000 kcal metabolizable energy) and 4 others had less than the RA (1.67 to 1.92 vs 1.98 g/1,000 kcal metabolizable energy). Another milk replacer contained more than the RA for arginine but less than the RA for methionine and cystine (1.08 vs 1.75 g/1,000 kcal metabolizable energy) and tryptophan (0.49 vs 0.58 g/1,000 kcal metabolizable energy). Ten of 15 milk replacers were supplemented with purified L-arginine; of the 5 milk replacers that contained less than the RA for arginine, 2 were supplemented and 3 were not supplemented. The milk replacer with the lowest concentration of methionine and cystine included DL-methionine in the ingredient list.

Total fat concentration was less than the range of fat concentrations in dog milk in 7 of the 15 milk replacers (Table 3; **Figure 1**). Three milk replacers contained concentrations of the essential n-6 fatty acid linoleic acid that were approximately half the minimum value detected in dog milk, whereas 2 other milk replacers contained approximately 70% more linoleic acid than the maximum value detected in dog milk. One milk replacer contained less ALA (18:3 n-3) than the lowest value in dog milk; 5 milk replacers contained more ALA than the highest value in dog milk (**Figure 2**). All milk replacers contained concentrations of ARA less than the lowest value for dog milk, and 14 of 15 milk replacers had ARA concentrations less than the NRC RA. Only 3 milk replacers contained detectable concentrations of both EPA (20:5 n-3) and DHA (22:6 n-3); all 3 products listed fish oil or cod liver oil in the ingredient list. Only one of these milk replacers had EPA and DHA concentrations within or higher than the range in dog milk. Omega-6:n-3 ratios were 6.15 (range, 4.46 to 7.56) for milk samples and 6.05 (range, 2.01 to 35.86) for milk replacers.

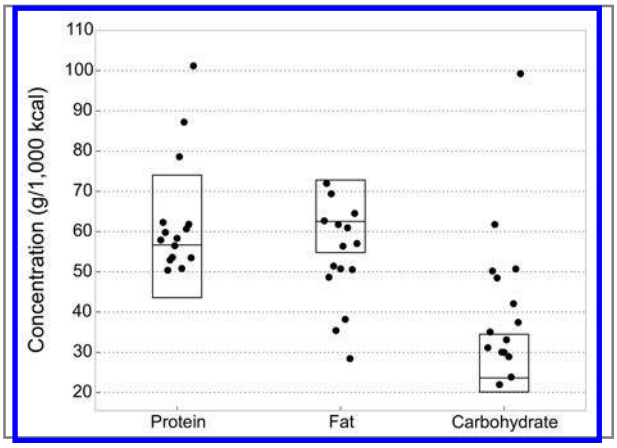

Figure 1—Protein, fat, and carbohydrate concentrations (g/1000 kcal gross energy basis) of 5 dog milk samples (box plots) and 15 milk replacer samples (black circles). The horizontal line in the box plot represents the median dog milk value, and the box represents the range.

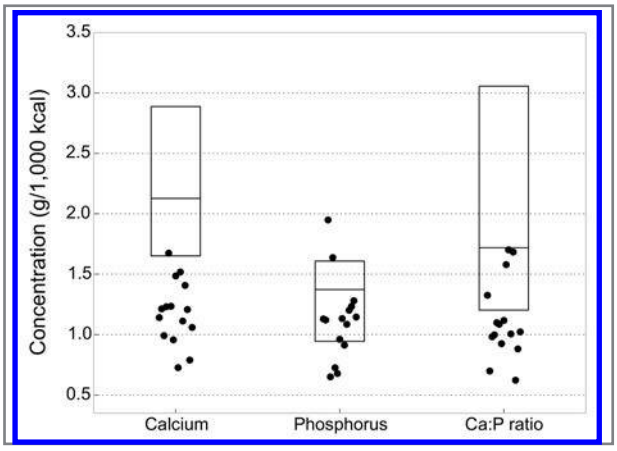

Figure 2—Calcium and phosphorus concentrations (g/1,000 kcal gross energy basis) and calcium-to-phosphorus ratios (Ca:P [unitless]) of 5 dog milk samples (box plots) and 15 milk replacer samples (black circles). The horizontal line represents the median dog milk value, and the box represents the range.

Total sugar concentrations in the 15 milk replacers ranged widely (Table 4). As in dog milk, most of the sugar in the milk replacers was in the form of lactose; however, all of the milk replacer samples contained more glucose than did dog milk, and 6 of the 15 milk replacers contained measurable fructose, which was detected in only low concentrations in 2 dog milk samples. Two milk replacers contained lactose concentrations 289% and 193% greater than the highest concentration in the 5 dog milk samples.

Nine of 15 milk replacers contained phosphorus concentrations within the range in dog milk, but only

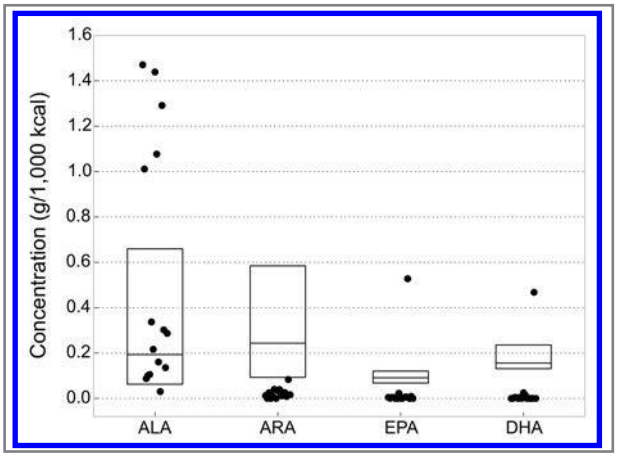

Figure 3—Fatty acid concentrations (g/1,000 kcal gross energy basis) of 5 dog milk samples (box plots) and 15 milk replacer samples (black circles). The horizontal line represents the median dog milk value, and the box represents the range.

1 milk replacer had a calcium concentration within the range in dog milk, with the other 14 calcium concentrations being lower (**Figure 3**; Table 3). Most milk replacers (11/15) had calcium-to-phosphorus concentration ratios less than the range for dog milk, and 9 of 15 had ratios < 1.1, which is generally considered to be the minimum appropriate ratio for growth of dogs.[25]

Although the intention was for 15 products to be purchased and analyzed, it appeared on close examination of the packaging after purchase and analysis that several of the products were the same formula, but with different branding. Four apparent sets of duplicates within the 15 milk replacer products were detected by noting identical guaranteed analyses, ingredient lists, and in some cases manufacturer address, lot numbers, plant codes, and other label information. Because this was a descriptive study of the nutritional content of the samples, all 15 samples were considered as separate observations in the main analysis. A set of duplicates[f,n] was determined to have the exact same lot numbers and date codes and had similar analyses, as did another set of duplicates.[b,m] Another set of duplicates[d,j] had the same plant code, and this set[d,j] and another set[a,c] had different lot numbers; these sets had variable results for nutrient analyses.

Discussion

Results of this study suggested that currently available puppy milk replacers contained variable nutrient concentrations and were not close matches to dog milk. Many products had potentially serious problems such as inadequate calcium, insufficient calcium-to-phosphorus ratio, low caloric density, inappropriate feeding directions, and excessive lactose concentrations. All of these concerns could contribute to poor puppy growth and viability.

It is also concerning that many manufacturers of the milk replacers chose to include standardized feeding directions on the basis of volume rather than adjusting for the caloric density of the individual products. Newborn puppies have been suggested[24] to require 25 kcal/100 g of body weight/d; however, 100-g puppies fed according to the package directions for the 15 milk replacers would receive 12.3 to 40.5 kcal of metabolizable energy/d. Especially for novice puppy raisers, adherence to the feeding directions for some of the products could easily lead to substantial over- or underfeeding. Compounding the issue is that even though several package directions recommend weighing the puppies regularly, none provide guidelines on appropriate rates of weight gain to ensure adequate intake.

Nutrient concentrations of the dog milk and milk replacers were compared on a gross energy basis rather than a metabolizable energy basis owing to the lack of published methods for estimating metabolizable energy for puppies prior to weaning. It is also not known whether the digestibility of the milk replacers is similar to that of dog milk, nor whether commonly used factors such as the modified Atwater factors of 3.5 kcal/g from protein and carbohydrate and 8.5 kcal/g used for pet foods or the standard Atwater factors of 4 kcal/g for protein and carbohydrate and 9 kcal/g from fat used for human foods are appropriate for puppies prior to weaning. Metabolizable energy can be anticipated to be lower than gross energy for both milk and milk replacers, but whether all are affected to the same degree cannot be estimated. However, owing to the wide variation in energy density, comparing the samples on an as-fed or even dry matter basis was more likely to lead to misinterpretation of the results. One consequence of the use of a gross energy basis instead of a metabolizable energy basis is that it can complicate comparison with NRC recommendations, which use metabolizable energy calculated on the basis of typical digestibility of pet food ingredients. On the basis of digestibility factors for human milk and the main ingredients of milk replacers (ruminant milk, corn syrup, and vegetable oils), both dog milk and milk replacers would be anticipated to have higher digestibility than typical pet food ingredients, suggesting that the use of the standard Atwater factors would be more appropriate than modified Atwater factors. Therefore, for the purposes of comparison with the NRC, milk replacers and milk sample data were converted to a metabolizable energy basis with standard Atwater factors. Further studies are needed to investigate the digestibility of milk and milk replacer formulas in puppies prior to weaning.

One of the milk samples contained less than the NRC RA for tryptophan, threonine, and lysine, but the deficiencies were slight and may have been within the margin of error of the assay. The milk replacers more commonly contained less than the RA for amino acids, and these differences typically could not be easily explained by assay variation, with the potential exception of 2 samples that were low in arginine. Two of the 5 low arginine values were within 10% of the RA and may have been within the margin of error of the assay; however, variation likely did not account for the other 3 values that were less than the RA. Arginine-deficient milk replacers have been reported to be associated with cataracts in wolf and large-breed puppies.[24] Considering that dog milk replacers are invariably based on ruminant milk, usually from cows or goats, which do not have a dietary requirement for arginine, it is not

surprising that many of the unsupplemented products contained borderline or inadequate arginine, compared with the RA. A previous study[1] found that half of the dog milk replacers analyzed had less arginine than did dog milk, although all the values met the NRC MR on a caloric basis and were not fed to puppies as part of that study. Another study[26] found cataracts in kittens fed a cat milk replacer that were presumed to have been associated with inadequate arginine concentration because the arginine concentration of the milk replacer was only 50% that of cat milk.

Long-chain PUFA are of increasing interest in developmental biology. The predominant PUFA found in the brain are DHA and ARA, and DHA is important for neural and retinal development. In the present study, all milk replacers had lower concentrations of ARA than did dog milk and all but 1 product had lower concentrations of EPA and DHA than did dog milk. Marked skin abnormalities have been reported in puppies fed diets deficient in linoleic acid, which subsequently led to low whole-body concentrations of both linoleic acid and ARA.[27] This fatty acid also has an important role in early mammalian development.[4] Studies in premature human infants have associated low blood concentrations of ARA with increased morbidity rates[28] and reduced growth in the first year after birth.[29] Long-chain n-3 PUFA in dogs have been recently studied; puppies that consume milk from dogs fed diets supplemented with long-chain n-3 fatty acids (EPA and DHA) and are then weaned onto the mothers' diet had better visual acuity at 12 weeks of age than puppies fed a nonsupplemented control diet.[30] Results of other studies[31,32] suggest that puppies fed a DHA-supplemented diet after weaning had faster learning, improved cognition, and better memory than puppies fed unsupplemented diets. Therefore, it is reasonable to assume that puppies fed a milk replacer deficient in DHA and ARA may have deficits in neural, retinal, and overall development in comparison to puppies raised on dog milk. Studies to confirm this assumption have not yet been performed.

In dogs, concentrations of PUFA in milk generally reflect dietary concentrations.[33] Therefore, to provide the most reasonable comparison possible for the milk replacer formulas, we specifically excluded dogs that were eating diets deemed to be heavily supplemented with n-3 fatty acids (> 1.5 g/1,000 kcal of total n-3 fatty acids, including ALA). The inclusion of ALA was a concession to the fact that not all of the manufacturers of the diets being fed to the donor dogs were able or willing to provide data for DHA, EPA, or ALA separately, but all could provide the total concentration of n-3 fatty acids. Supplementation of female dogs with ALA during gestation and lactation does not result in increased amounts of DHA in canine milk.[33,34]

The NRC has published RAs for ALA, for DHA and EPA combined for all life stages of dogs, and for ARA for growing puppies. The Association of American Feed Control Officials, which publishes nutrient profiles that are used as guidelines by manufacturers for formulating commercial dog foods, does not provide minimal concentrations of any of these PUFA.[25] As such, in the authors' experience, DHA and EPA concentrations in diets marketed as appropriate for reproduction and growth range from undetectable to in excess of the NRC safe upper limit of 2.8 g/1,000 kcal. Despite selecting female dogs consuming lower concentrations of total n-3 fatty acids, milk from all 5 female dogs in this study was higher in DHA and EPA than all but 1 milk replacer. It is likely that many female dogs eating diets that are higher in PUFA have higher concentrations in their milk, leading to an even greater disparity, compared with results for bitches fed milk replacers. Although most of the milk replacers contained similar or higher ALA concentrations, compared with dog milk, ALA supplementation of both gestating dogs and growing puppies does not have equivalent benefits to supplementing with DHA directly when it comes to retinal function; therefore, having similar concentrations of ALA in milk replacers, compared with dog milk, may be of little value, compared with similar concentrations of EPA and DHA.[34] More data are needed, but current research suggests that puppies may benefit from supplementation of DHA (but not necessarily ALA) to at least the concentrations found in the milk of domestic dogs consuming diets lower in total n-3 fatty acids.

Although the importance of DHA and ARA in neonatal development has only recently been recognized, linoleic acid (18:2 n-6) has long been known to be an essential fatty acid with important roles in skin integrity among other body systems. Linoleic acid competes for the same enzymes as ALA, and the downstream long-chain n-6 fatty acids such as ARA are considered to promote the production of more inflammatory end products (ie, eicosanoids) than are the n-3 family.[35] Thus, even though there is an essential requirement for linoleic acid, high amounts are thought to drive excessive inflammation, particularly in the absence of appropriate amounts of n-3 fatty acids. Two of the milk replacers had linoleic concentrations far in excess of that found in the dog milk, and some had n-6:n-3 fatty acid ratios as much as 5-fold that of dog milk. Both of these factors could potentially contribute to a greater propensity for inflammation in puppies. Interestingly, 2 dog milk samples also contained linoleic acid concentrations that were much less than the NRC RA. Previous studies that have reported linoleic acid concentrations in dog milk have not provided adequate information to allow for a direct comparison with the values obtained in this study. An NRC MR for growing animals is not available for linoleic acid[24]; however, in previous studies,[36,37] weaned puppies had dermatologic signs consistent with linoleic acid deficiency after 2 to 3 months if not fed diets containing at least 2% linoleic acid (approx 2.2 g/1,000 kcal of metabolizable energy). Because most puppies are introduced to balanced diets containing at least 2.9 g of linoleic acid/1,000 kcal (the Association of American Feed Control Officials minimum recommended concentration for growth and reproduction) at around 4 weeks of age and are typically completely weaned after 6 to 8 weeks, it may be that dog milk does not require such high concentrations to sustain appropriate puppy growth and skin condition in the short period of time that puppies rely only on dog milk for nutrition. Alternatively, it may also be that younger puppies have lower linoleic acid needs than puppies after weaning or that the concentration of oth-

er n-6 fatty acids in dog milk (eg, ARA) has a sparing effect on linoleic acid. Finally, the possibility of incomplete recovery of linoleic acid from samples or different processing methods could also not be entirely ruled out to explain the discrepancy between linoleic concentrations in dog milk versus the NRC requirements.

Two goat milk–based milk replacer products (one was 100% goat milk) from the same manufacturer had a high concentration of lactose, compared with dog milk. Undigested lactose and other sugars in the intestine have high osmolarity and can lead to osmotic diarrhea, which can quickly dehydrate a neonate. Two other goat milk–based products from a different manufacturer had concentrations of lactose within the range of dog milk, so it cannot be assumed that all goat milk–based products contain a high concentration of lactose. Somewhat surprisingly, other than the excessive lactose concentration, the 100% goat milk was a better match for dog milk than some of the other milk replacers; however, it cannot be recommended because other tested products were more similar to dog milk and contained less lactose.

Most milk replacers had less calcium than the dog milk analyzed in this study. This finding is in contrast to findings of a recent European study[2] that measured calcium and phosphorus concentrations in 8 dog milk replacers; most formulas in that study[2] had similar or greater calcium concentration than did dog milk (for the milk replacers, 1.69 to 2.47 g/1,000 kcal for calcium and 1.18 to 1.66 g/1,000 kcal for phosphorus), with all products having calcium-to-phosphorus ratios > 1.2 Another previous study[1] that evaluated 8 dog milk replacers found that most milk replacers had more calcium and phosphorus than did dog milk, although a wider range of calcium and phosphorus concentrations (1.14 to 3.09 g/1,000 kcal and 1.2 to 2.8 g/1,000 kcal, respectively) was found. Only 1 product in that study[1] had a calcium-to-phosphorus ratio < 1. Possible explanations for the higher amounts of both minerals in milk replacers in those studies,[1,2] compared with the present study, may be differences in methodology, products tested (product names were not always provided), or methods of determining energy density. Additionally, calcium (and to some extent phosphorus) concentrations in dog milk generally increase up until about day 35 of lactation,[38] which also may account for some of the difference in these minerals in dog milk among the studies.

Most dog milk samples also contained less than the NRC MR or RA for calcium and phosphorus. Whereas 1 milk sample calcium value that was less than the MR was likely within the margin of error of the assay, the other low calcium values could not be explained by assay variation alone. Another study[38] also found dog milk calcium and phosphorus concentrations that were less than the NRC recommendations. The mean milk calcium and phosphorus concentrations (1.32 and 0.86 g/1,000 kcal, respectively) in that study[38] were even lower than those in the present study. Indeed, it has been reported that calcium and phosphorus concentrations in dog milk are insufficient to allow maximal bone growth.[3] These data indicate the potential need for dietary supplementation even in dog milk–fed puppies, particularly those that are critically ill. However, growth problems have been reported with large-breed puppies fed high amounts of calcium. On the basis of data from the present study, the bigger concern is that nearly all of the milk replacer samples analyzed contained less calcium than did dog milk; no milk replacers contained more calcium than dog milk.

The present study was limited by the fact that only 1 sample of each product was assayed because of the expense of the analyses. Therefore, the consistency of these products within or across lots was not known, and results may have been different had the products been purchased at a different time. When 2 products from the same lots were inadvertently analyzed (owing to several sets of apparent duplicates), the values were quite similar, suggesting minimal variation within lots for those products. However, the 2 product pairs for which 2 lots were inadvertently analyzed were dissimilar in nutrient content. This suggests that at least for these 2 manufacturers, there was a moderate amount of variability among lots. Besides inter- and intralot variation, several of the products were available in both powdered and liquid forms. We chose to assay more products overall, rather than include both dry and liquid forms of fewer products; it remains unclear whether both forms share the same nutrient profile. A previous study[1] compared the proximate analyses and some essential minerals between 2 dog milk replacers in both dry and powdered form reconstituted per the manufacturers' directions and found some substantial nutrient differences, with some nutrients varying by up to 4-fold when adjusted for energy density. Therefore, it cannot be assumed that the liquid or dry versions of the products tested in this study would necessarily have similar nutrient profiles and similar performance. Full nutritional analyses of all essential nutrients were also not performed in this study because of cost considerations; therefore, it is possible that formulas that appeared to provide adequate amounts of the essential nutrients that were assayed could be deficient in other essential minerals or vitamins.

Exact nutrient requirements for puppies between birth and 4 weeks of age are not known, but it is assumed that in this period before solid foods are introduced, dog milk from a healthy, well-nourished dog should provide adequate nutrient concentrations and calories for growth of a typical size litter until weaning. The NRC has published an MR and RA for protein and amino acids for puppies from 4 to 14 weeks of age. For fats, vitamins, and minerals, the NRC makes recommendations only for growing puppies > 14 weeks of age. Minimal requirements are typically based on studies that use purified diets in weaned puppies, whereas the RA include safety margins to reflect the bioavailability of nutrients in typical pet food ingredients. As such, these values may not accurately reflect requirements for growth in unweaned puppies fed dog milk exclusively and may help explain why some of the dog milk samples in this study did not meet or exceed the NRC MR and RA for all essential nutrients.

Further research is needed to determine the appropriate nutrient requirements and ideal nutrient composition of milk replacers for young puppies. However,

results of this study supported the hypothesis that currently available milk replacers have lower concentrations of long-chain PUFAs than does dog milk. Many products could be made more similar to milk by the addition of long-chain PUFA, arginine, calcium, and, depending on the individual product, other nutrients. In the meantime, dog owners and breeders should be counseled that not all milk replacers are the same and products with serious nutritional concerns should be avoided.

a. Nutri-Cal Milk Replacer powder, Vétoquinol USA Inc, Fort Worth, Tex.
b. Just Born Highly Digestible Milk Replacer Liquid Formula for Puppies, Farnam Co Inc, Phoenix, Ariz.
c. Pro-Biolac For Puppies powder, Vet Solutions LP, Fort Worth, Tex.
d. Espilac Puppy Milk Replacer Liquid, PetAg Inc, Hampshire, Ill.
e. puppylac Milk Replacer for Puppies powder, Glo-Marr Products Inc, Lawrencebug, Ky.
f. Goats Milk Esbilac for Puppies Liquid, PetAg Inc, Hampshire, Ill.
g. Precision nutrition milk replacer for puppies liquid, Hartz Mountain Corp, Secaucus, NJ.
h. Goat-A-Lac powder, Thomas Laboratories, Tolleson, Ariz.
i. Mother's Milk Canine and Feline Food Supplement powder, Wysong Corp, Midland, Mich.
j. Ultra Mega Premium Milk Replacer Enriched Liquid Formula, General Nutrition Corp, Pittsburgh, Pa.
k. Milk Replacer powder, 21st Century Pet Nutrition, Tempe, Ariz.
l. Goat's Milk G.M. powder, Thomas Laboratories, Tolleson, Ariz.
m. Nuturall-C Puppy liquid, Veterinary Products Laboratories, Phoenix, Ariz.
n. Ultra Mega Premium Milk Replacer Goat's Milk Liquid, General Nutrition Corp, Pittsburgh, Pa.
o. Foster Care powder, Breeder's Edge, Orange City, Iowa.
p. Nutrition Laboratory, Smithsonian National Zoological Park, Washington, DC.
q. Model 2400, PerkinElmer Inc, Norwalk, Conn.
r. Beckman DU model 640, Beckman Coulter Inc, Fullerton, Calif.
s. Model 800 Perkin Elmer Analyst Flame/Furnace Atomic Absorption Spectrophotometer, Perkin Elmer Co, Waltham, Mass.
t. MRX TC Revelation, Dynex Technologies Inc, Chantilly, Va.
u. HP Series II 5890 gas chromatograph HP-5971 mass spectrometer, Hewlett-Packard Co, Palo Alto, Calif.
v. Supelcowax SP-10 capillary column, Sigma-Aldrich Co, St Louis, Mo.
w. Speedvac SVC200H Concentrator, Savant Instruments Inc, Farmingdale, NY.
x. Millipore Millex–FH, EMD Millipore, Billerica, Mass.
y. Biochrom 30, Biochrom Ltd, Cambridge England.
z. NP Analytical Laboratories, St Louis, Mo.
aa. Minitab, version 16, Minitab Inc, State College, Pa.

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