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Is Extended Lactation Nutritionally Important for the Weaning of Wild Toque Macaques, *Macaca sinica*? Evidence From Milk Composition

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ABSTRACT

Successful lactation is important to offspring survival in wild primates, both during infancy and the transition of weaning. We examined changes in the composition of milk of wild toque macaques to assess its nutritional role both during primary lactation (< 7 mo) and subsequent infant care. We collected 72 milk samples from wild toque macaques (*Macaca sinica*) in Polonnaruwa, Sri Lanka, up to an infant age (iAge) of 2.2 y and assayed them by standard methods as developed at the Smithsonian's National Zoo. During the first 7 mo *M. sinica* milk contained on average 86.1% water, 13.9% dry matter, 4.25% fat, 1.62% protein, 7.84% sugar, 0.792 kcal/g energy, 0.083% calcium, 0.026% phosphorus, 0.012% magnesium, 3.32 ppm zinc, 18.1 mM/kg H_2O sodium, 4.01 mM/kg H_2O potassium and 16.2 mM/kg H_2O chloride. Dry matter, fat, and energy increased significantly during this period, but much greater changes were observed in these and other constituents (including protein, phosphorus, and zinc) during the subsequent supplemental lactation period. The average water (74.0%), and sugar (6.31%) decreased, whereas dry matter (26.0%), fat (15.9%), protein (3.8%), energy (1.91 kcal/g), phosphorus (0.050%) and zinc (11.9 ppm) concentrations of *M. sinica* milk during the supplemental period are higher than any prior reported values for primate milks. As infants aged and transitioned to independent feeding the nutrient composition of milk changed by reducing water and sugar that older nursing offspring obtained progressively more from water and fruit in the environment, rather than from milk. We hypothesize that this switch in sourcing water and sugar conserves maternal energy, nutrient balance, and fitness. Notwithstanding, supplemental lactation continued to provide other nutrients (including fat, protein, energy, P, and Zn) that may serve an important nutritional/developmental function over and above maintenance of maternal-infant bonds, at least in wild primates.

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1 | Introduction

Lactation is the costliest phase of mammalian reproduction; the quantities of energy and nutrients provided to the young as milk far exceed cross-placental transfers (Oftedal 1985). There is great taxonomic variation in milk composition, in the intensity and duration of lactation, and in the weaning process by which the young transition from milk dependence to independent feeding (Langer 2003; Oftedal 1984; Oftedal and Iverson 1995). Most efforts to understand phylogenetic variation and biological correlates of milk composition and yield have focused on peak lactation, defined as that period of high milk yield before substantial consumption of solid foods by the young (Hinde and Milligan 2011; Oftedal 1984; Oftedal and Iverson 1995; Riek 2011; Skibiel et al. 2013). A perhaps unintended consequence of this focus has been to overlook significant changes in milk composition that occur as the young become more self-reliant in feeding, and that may reflect an adaptive process as milk transitions from the primary source of nutrients to a nutritional supplement. As the benefit of any supplement depends on nutritional inadequacies in the overall diet, natural selection should favor the secretion of milk in this supplemental period that is matched to shortfalls in weaning diets.

Studies of milk composition in primates have been conducted exclusively on captive and well-fed primates that exhibit an array of life history parameters whose values differ markedly compared to those of wild primates that manifest longer interbirth intervals, higher infant mortality, slower offspring growth, lower adiposity, higher age of menarche, and later age of weaning (reviewed in Dittus and Baker 2024). The subjects of this study, toque macaques (*Macaca sinica*), too, show comparatively higher hematological indices of dehydration, injury, and infection (Ekanayake et al. 2003) than captive macaque species. The aim of this study was to test hypotheses, outlined below, by analyzing the changes in the composition of milk in wild toque macaques whose females supplement their offsprings' diets with milk during self-reliant feeding for a year or more beyond primary lactation (Dittus and Baker 2024). Prolonged supplementary lactation manifests a stark contrast to the early weaning observed in well-fed managed primates (Langer 2003).

1.1 | Lactation, Weaning, and Reproduction in Wild Toque Macaques

At Polonnaruwa, Sri Lanka, female toque macaques were briefly captured and their mammae were manually examined for the presence or absence of milk among females with known aged infants (Dittus and Baker 2024). It was determined that all mothers had milk for infants less than 7.2 months old; thereafter the number of lactating mothers decreased gradually with increasing infant age but extended up to 24 months for a few. These findings on the availability of milk were integrated with behavioral profiles relevant to weaning as revealed by a 5-year study of mother-infant relationships in the same population (Dittus and Baker 2024).

The combined data pointed to firstly, a gradual decline in nursing frequency from birth to 16 mo infant age, and secondly, a pivotal change in the mother-infant relationship at about 7 months postpartum. This was marked by (i) a peak in the rates of maternal rejection of infants' nursing attempts, (ii) a near cessation of infant carrying by mothers, (iii) infants foraging at rates typical of older (> 1 yr) offspring. A second peak in maternal rejection occurred as a final weaning at 14 to 20 months. The timing of the bimodal rejection peaks coincided with those in the resumption of sexual cycling among lactating mothers and their subsequent new pregnancy.

In the study by Dittus and Baker (2024), nipple stripped milk ($n = 142$) was assessed subjectively as to either dilute and watery or thick and creamy; it indicated a progressive transition from dilute to dense milk with infant age (Figure 1), suggesting a reduction of milk water content with extended lactation. Here we report on the changes in the composition of all 15 major constitutes of milk with infant age, based on laboratory analyses of 72 milk samples.

1.2 | Weaning Age in Other Primates: Early Notice and Shutting Down

It is intriguing that a modal weaning age has been calculated as 7 months for 16 species of cercopithecines that have been managed under well-fed conditions (Langer 2003). Bimodal weaning peaks, as in wild toque macaques, have not been observed in captive primates. However, comparable ones have been observed in a variety of other primates living under natural conditions (reviewed in Dittus and Baker 2024).

1.3 | Extended Lactation Promotes Infant Survival

We interpret these data in the toque macaque and other cercopithecines to indicate that milk acquired via suckling is the predominant source of nutrients up to 7 months (the primary
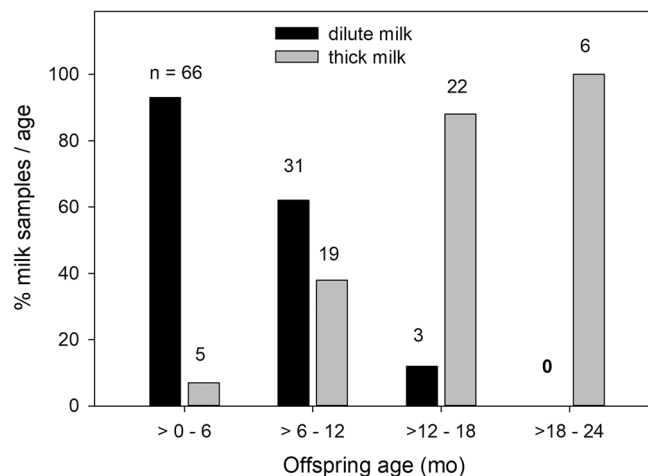

FIGURE 1 | Subjective assessments of the density of nipple-stripped milk (dilute and watery, or thick and creamy) at the time of sampling ($n = 146$) in relation to offspring age (unpublished from Dittus and Baker 2024).

lactation period) but that a second phase (≥ 7 months) involves milk intake as a supplemental source of nutrients given that self-acquired foods have become the predominant source of energy and nutrients. From the perspective of maternal physiology and infant nutrition, we term this phase the supplemental lactation period. At a theoretical level, the selective pressures driving the evolution of milk nutrient content may be different for a supplemental food as mammary secretion needs to complement, rather than substitute for eaten foods. We propose that the supplemental lactation period is key to bridging a developmentally difficult phase for infants, when energetic and nutritional needs are high but may be difficult to access in natural environments. In toque macaques, for example, the youngest juveniles are disadvantaged in food competition with older higher-ranking group members and feed at the slowest rates, on the poorest quality foods, and die at higher rates (Dittus 1977a). Extended lactation slowed reproduction and promoted infant survival in toque macaques (Dittus and Baker 2024), rhesus (*Macaca mulatta*) (Lee et al. 2019), mountain baboons (*Papio cynocephalus*) (Lycett et al. 1998), and humans (Wang et al. 2022).

1.4 | Milk Composition in Cercopithecines

The evolution of milk as a nutritive secretion has a long history dating back to evolving synapsids in the Carboniferous (Oftedal 2002; Oftedal and Dhouailly 2013). Milk production involves many evolutionary novelties, including 1. Large, complex casein micelles stabilized by Ca and P; 2. milk-specific whey proteins such as α-lactalbumin and β-lactoglobulin; 3. uniquely synthesized membrane-bound milk fat globules, and 4. novel sugars not found elsewhere in nature, including lactose and lactose-based oligosaccharides (Oftedal 2013). These constituents are universal to mammalian milks, except where genetic modifications have resulted in pseudogenes that no longer are transcribed and translated, and mammalian species adjust the composition of milk by altering relative secretion rates of these constituents. The nutritional contributions of the various constituents are complementary, as when caseins augment milk Ca content beyond what is possible by solution (Holt et al. 2009; Smyth et al. 2004), whey proteins supply limiting sulfur amino acids important to offspring growth (Oftedal et al. 2014), and Fe-, Cu- and Zn-binding proteins complement trace elements associated with other milk constituents (Hurley 1985; Krebs et al. 1995).

Among primates, the milks of cercopithecines are relatively well studied, including a *Chlorocebus*, a *Cercopithecus*, 3 species of baboon (*Papio* spp., but data were not reported separately) and 5 species of *Macaca* (Table 1). Note that these only represent the primary lactation period and food-provisioned females (Table 1). Thus, the issue of milk composition during the important supplemental lactation period has not been previously addressed.

1.5 | Aims and Hypotheses

We report here on the major energetic constituents (fat, protein, sugar, energy), mineral elements important to growth and bone formation (Ca, P, Mg, Zn) and major electrolytes (sodium [Na], potassium [K], and chloride [Cl]) of toque macaque milk. In contrast to prior cercopithecine studies, milk was sampled from animals in the wild and during both the primary and supplemental lactation periods. To date, the only published data on toque macaque milk are based on a few samples of milk we provided to L. Milligan and T. Urashima for comparative studies of milk fatty acids and milk oligosaccharides (Goto et al. 2010; Hinde and Milligan 2011; Milligan et al. 2008; Taufik et al. 2012). We sought to test four hypotheses: 1. That milk from wild toque macaques differs in energy content and nutrient composition from the milks of other, food-provisioned cercopithecines; 2. That milk produced during primary and supplemental lactation periods of wild toque macaques differ in energy and nutrient composition; 3. That milk collected during the supplemental lactation period is more than just a terminal secretion reflecting mammary gland involution and thus may be significant in terms of primate natural history evolution; 4. That the composition of milk is adapted to changes firstly, in the nutrient requirements of offspring from milk as they develop independent feeding and drinking and secondly, in maternal ability to provide those nutrients.

2 | Methods

The methods of field observation, capture and release of macaques, and milk sampling comply with all regulations regarding the ethical treatment of research subjects as prescribed by the National Institute of Fundamental Studies, Sri Lanka, and the Smithsonian's National Zoo and Conservation Biology Institute, USA, and adhered to the American Society of Primatologists principles for the ethical treatment of primates. The methods applied, the metadata, and raw data content files relevant to this publication have been reposited for public access (Dittus et al. 2025).

2.1 | The Study Population Ecology and Milk Collection Procedures

Toques macaques are relatively small (5.5 kg adult males, 3.1 kg adult females), long tailed, and endemic to Sri Lanka. They have been studied in dry-evergreen forest in the Nature Sanctuary and Archeological Reserve at Polonnaruwa continuously for over five decades (1968–2025). Salient aspects of their seasonal forest environment, diet, ecology, behavior, anatomy, and demography have been abstracted in Dittus and Baker (2024). Their population size is food limited showing near zero population growth in undisturbed natural environments, but the introduction of anthropogenic foods has stimulated population growth in affected groups (Dittus et al. 2019). Seasonal scarcity of water sources affects all groups' range ecologies and limits the species' geographic distribution (Dittus 1977b); drought leads to hematological dehydration (Ekanayake et al. 2003) and increased mortality especially among infants and juveniles (Dittus 1977a). The standing population for this study involved more than 1,000 individuals distributed among 39 independent social groups. Of these, all parous females of 17 groups were sampled for milk collection during five different years (1986 to 1987 and 1992 to

TABLE 1 | Comparison of cercopithecine milks[a].

		Infant age (days)	N	Dry matter %	Fat %		Crude protein %		Total sugar %		Gross energy kcal/100 g	Percent energy from:			Ref[b]
												Fat %	Protein %	Sugar %	
1. Vervet monkey	B	~90–120	4	—	3.1	F	1.6	D	8.5	H	71.2	40	13	47	1
Chlorocebus pygerythrus															
2. Talapoin monkey	B	17–38	5	12.1	2.8	G	2.0	N	7.3	R	66.2	39	18	43	2
Cercopitheus talapoin	B	178–192	8	15.1	4.3	G	2.4	N	8.0	R	84.9	47	16	37	2
3. Baboons	B	21–70	30	—	4.6	C	1.5	N	7.8	R	81.7	52	11	38	3
Papio spp.	B	36–279	42	14.4	5.0	G	1.6	N	7.3	R	83.8	54	11	34	4
Papio anubis	B	47–129	8	13.6	4.7	G	1.6	N#	7.3	P	81.0	52	12	36	5
4. Crabeating macaque	B	44–119	8	12.7	5.2	G	1.7	N*	4.8	X	76.8	62	13	25	6
Macaca fascicularis															
5. Japanese macaque	B	28–105	9	14.7	4.9	F	1.8	L	5.1	Z	75.3	59	14	27	7
Macaca fuscata	B	119–196	9	24.7	11.7	F	3.4	L	5.2	Z	147	73	13	14	7
6. Rhesus macaque	B	29–39	58	—	4.6	G	1.8	N	7.6	P	82.5	51	13	36	8
Macaca mulatta	B	91–118	58	—	6.2	G	2.1	N	7.5	P	98.4	57	13	30	8
Macaca mulatta	B	15–92	6	13.5	4.1	G	1.7	N#	7.7	P	78.0	48	13	40	5
7. Pig-tailed macaque	B	70–98	5	16.1	6.7	G	1.8	N#	7.6	P	102.0	60	11	30	9
Macaca nemestrina															
7. Bonnet macaque	B	57–133	14	—	2.5	C	2.2	N	7.5	Z	65.0	35	20	45	10
Macaca radiata	B	151–216	10		7.0	C	2.7	N	7.5	Z	109	58	14	27	10
8. Toque macaque	W	15–120	17	13.2	3.5	G	1.7	N	8.0	P	73.1	44	13	43	11
Macaca sinica	W	128–219	15	14.8	5.1	G	1.6	N	7.7	P	86.0	54	11	35	11

[a]Milk samples obtained from breeding colonies (B) or wild populations (W) during primary lactation period, with results for mid lactation in bold. Method of analysis is indicated by letter following numerical value: C - creamatocrit; D - Dumas; F - Folch; G – Roese-Gottlieb; H - high performance liquid chromatography; L - Lowry; N – Kjeldahl (total nitrogen); N* - Kjeldahl (protein nitrogen); N# - CHN gas analysis (total nitrogen); P - phenol-sulfuric acid; R - reducing sugar; S - spectrophotometric; X - by difference; Z – enzymatic.

[b]References: 1. Osthoff et al. 2009; 2. Buss and Cooper 1970; 3. Roberts et al. 1985; 4. Buss 1968; 5. Glick et al. 2021; 6. Nishikawa et al. 1976; 7. Ōta et al. 1991; 8. Lönnerdal et al. 1984a; 8. Hinde et al. 2009; 9. Glick and Power 2023; 10. Laudenslager et al. 2010; 11. This study.

1994), yielding 72 milk samples. All macaques were individually identified by their natural markings and tattoos (National Research Council 1981), and their life-histories were monitored from birth to death (Dittus 2004). The chronological ages of all juvenile and most adult individuals in this sample were based on known birthdates; those of a few adults, that were born before 1968, were estimated from morphological development; females are physically mature or "adult" by 6 years old, and males by about 9 years old (Cheverud et al. 1992).

Procedures for the capture and release of macaques have previously been described (Hoelzer et al. 1994). Macaques were habituated to enter steel mesh box traps (ca. $1 \times 1 \times 1.5$ m), which were baited with food for several days before the capture date. Upon capture, the mothers and their young were separated, and the mother tranquilized by IM injection of ketamine hydrochloride (Ketalar, Park-Davis Co). Macaques were released, watered, and fed after their full recovery from the anesthetic, usually within hours after sedation. Before milk collection, hair in the vicinity of each nipple was removed by shaving, and the nipple area cleaned with distilled water and dried. Females were injected IM with 0.20 cc (4 IU) oxytocin to induce milk let down. Milk was obtained by manual expression into 1.8 ml cryovials (1–10 cryovials per female, i.e., about 1.5–16 ml); an effort was made to evacuate the mammary glands as fully as possible. Milk samples in sealed cryovials were immediately submerged in liquid nitrogen for storage and transport from Sri Lanka to Washington, DC, USA. Milk was kept frozen at −20°C or colder throughout storage at the Smithsonian's National Zoo, and was assayed within a year of field collection. Milk samples were analyzed in this study from the 72 captured females whose infants ranged in age from 0.04 to 2.20 y. Of these capture events, three were repeat captures of the same mother but with different infants in separate lactation cycles.

2.2 | Laboratory Analysis

Frozen samples were thawed quickly by immersion of cryotubes in 50°C water, vortexed and immediately subsampled. Of the many cryovials of milk collected from each female, only one vial was assayed per female (or capture event), although in some cases samples from right and left nipples were pooled to give sufficient volume for analyses. We also analyzed sequential samples from one nipple of 5 females. All assays were conducted in duplicate or triplicate.

2.3 | Dry Matter (DM) and Water

Subsamples of 100 µL were pipetted into aluminum pans, weighed, dried for 3 h at 100°C in a forced convection oven, cooled in a desiccator and reweighed. Water was calculated as 100-DM%.

2.4 | Fat

Subsamples of 100–500 µL were pipetted into 12 mL ether-washed glass centrifuge tubes, and subject to sequential extractions with ammonium hydroxide, ethanol, petroleum ether, and diethyl ether in a micro-adaptation of the Röse-Gottlieb method (Helrich 1990, method 905.02). Steps were as described in Helrich (1990) except that sample and reagent volumes were one-tenth of those specified. Tubes were vortexed after each addition and centrifuged at 2500 rpm to separate layers at the end of each round of extraction. Extracted fat was removed from aluminum collection pans with warm petroleum ether, dried, and weighed.

2.5 | Crude Protein

Total nitrogen (TN) content was determined by Nessler and microKjeldahl procedures (Hood et al. 2009; Koch and McMeekin 1924). In the former, 30 µL subsamples were weighed, digested for 30 min with 0.4 mL concentrated sulfuric acid in a block heater at 150°C, flamed sequentially after additions of 30% hydrogen peroxide, reacted with Nessler's reagent (71.5 g sodium hydroxide, 25 g mercuric iodide, 20 g potassium iodide diluted to 1 L in distilled water), diluted with distilled water and read at 500 nm in a UV-visible spectrophotometer (Spectronic 1201, Milton Roy Co., Ivyland, PA). Standard curves were prepared with ammonium sulfate. In the micro-Kjeldahl procedure, 200 µL subsamples were digested with concentrated sulfuric acid, potassium sulfate, and a copper catalyst for 1 h after clearing of digests. Sodium hydroxide and distilled water were added, and ammonia was steam distilled into 4% boric acid containing bromocresol green and methyl red as indicators. N content was determined by titration with 0.0100 N hydrochloric acid. Both procedures were validated against standard macro-Kjeldahl procedures using cow's milk and National Institute of Standards (NIST) skim milk powder (Oftedal et al. 2014). Crude protein was calculated as TN x 6.38.

2.6 | Total Sugar

Sugar content was determined by the phenol-sulfuric acid colorimetric method (Hood et al. 2009; Marier and Boulet 1959) using standards prepared with lactose monohydrate. 15–20 µL subsamples were weighed, diluted with distilled water, mixed with phenol and concentrated sulfuric acid, vortexed, and after exactly 10 min transferred to a water bath at room temperature to terminate the reaction. Samples and standards were read at 490 nm in a UV-visible spectrophotometer (Spectronic 1201, Milton Roy Co., Ivyland, PA). Results were corrected to an anhydrous lactose basis.

2.7 | Gross Energy

Gross energy was calculated from fat, crude protein and sugar by a formula adapted from Oftedal (1984) and Perrin (1958): GE (kcal/g) = (9.11*Fat% + 5.86*protein% + 3.95*sugar%)/100. A subset of 13 toque macaque milk samples were also assayed directly by drying 100 mg milk onto 10 mg ether-washed cotton for 2 h at 95°C (Oftedal et al. 2014) and ignition in a Phillipson microbomb calorimeter (Hood et al. 2009).

2.8 | Minerals by Atomic Absorption Spectrophotometry

Samples were dried at 100°C in a forced convection oven and ashed overnight at 450°C in a muffle furnace. The ash was dissolved in 6 N hydrochloric acid and diluted with distilled deionized water. For sodium (Na) and potassium (K) analysis, lithium chloride (0.15% Li in final dilution) was added as a modifier and for calcium (Ca) and magnesium (Mg) analysis strontium chloride (0.5% Sr in final dilution) was added as a modifier; no modifier was used for zinc (Zn) analysis. These elements (Na, K, Ca, Mg, Zn) were measured by direct aspiration flame atomic absorption spectrophotometry (AAS) (Smith-Hieftje 12, Thermo Jarrell Ash Corp., Franklin, MA). Assay performance was validated against certified values for NIST skim milk powder (Standard Reference Material 1549): assayed AAS results averaged 101%, 103%, 99.2%, 100%, and 100% of certified values for Na, K, Ca, Mg, and Zn, respectively.

2.9 | Other Minerals

Phosphorus (P) content was determined by the photometric molybdovanadate or Gomorri method (Helrich 1990). Milk subsamples (0.25–0.5 g) were dried in a forced convection oven, ashed at 600°C for 4 h in a muffle furnace, solubilized in dilute hydrochloric and nitric acid, and diluted with distilled deionized water. The solution was mixed with molybdovanadate reagent and P content measured at 400 nm in a UV-visible spectrophotometer (Spectronic 1201, Milton Roy Co, Ivyland, PA). Standards were prepared with reagent grade KH_2PO_4. Assay of NIST milk powder averaged 101% of the certified value. Chloride (Cl) concentration was measured by silver ion titration by an automated coulometric-amperometric method using a digital chloridometer (Model 442-5000, Labconco, Kansas City, MO). Subsamples of 200–500 μL were acidified with 40% acetic acid/0.4 N nitric acid, diluted with distilled deionized water and inserted into the chloridometer with 4 drops of gelatin reagent (Labconco, Kansas City, MO). The coulometric titration with a pair of silver electrodes continued until the amperometric endpoint was reached. A 1000 mEq/L chloride standard solution was used for instrument calibration and assessment. Assay of NIST milk powder averaged 100% of the certified value.

Due to a shortage of milk sample volumes from ages 0.7 y to 1.1 y available material was prioritized for analyses of macronutrients leaving data gaps for other constituents (Figures 4 to 6).

2.10 | Statistical Analysis

2.10.1 | Sequential Milk Subsamples

To test whether there was an effect of sequential removal of milk samples on proximate composition, three sequential milk samples from the same animal, nipple and collecting event were compared by repeated measures analysis of variance, after testing for normality and constant variance.

2.10.2 | Bomb vs Calculated Energy

Caloric measurements by bomb calorimetry were compared to gross energy as calculated from fat, protein and sugar for the same samples by paired *t*-tests.

2.10.3 | Effect of Infant Age

The entire data set for each constituent was tested for normality and constant variance, and if the data conformed, were regressed against infant age (iAge) by incremental polynomial regression, adding linear, quadratic and cubic terms to a descriptive model as statistically significant ($p \leq 0.05$). Constituent data that did not conform to assumptions of normality and homoscedasticity were subject to logarithmic (ln) transformation before incremental polynomial regression. For one variable (protein) this transformation did not produce homoscedasticity, and therefore the relationship to iAge was examined by Spearman Rank Order Correlation. In all statistical analyses and results, iAge is expressed in year (y) units, but for ease of comprehension we discuss results relative to iAge in months, where a month is assumed to be of 30 d duration.

Our data span two periods of lactation, which we term primary lactation (iAge < 0.583 y or 7 mo) and supplemental lactation (iAge ≥ 0.583 y or 7 mo), that are represented by 34 and 38 milk samples, respectively, for a total of 72 samples. Mean values for these two periods were compared by *t*-tests if they passed both normality and equal variance tests; if not, they were compared by Mann-Whitney Rank Sum tests. Within each period, all milk variables were normally distributed and homoscedastic relative to infant age and could be described by linear regression; addition of quadratic terms were not significant when evaluated by incremental polynomial regression. Statistical analyses and graphing were performed using Sigmaplot/Sigmastat (SPSS Inc., Chicago, IL, USA).

3 | Results

3.1 | Methodological Tests

The totals of water, fat, protein and sugar accounted for 99.8% and 99.6% of milk in early-mid and late lactation, respectively (Table 2). For a subsample of milks ($n = 13$), calculated milk energy (kcal/g) was not significantly different from energy measured by microbomb calorimetry (calculated, $1.297 \pm SD$ 0.434; measured, $1.233 \pm SD$ 0.371; paired *t*-test, $t = -1.81$, $p = 0.10$). There was no effect of sequential sampling on DM, fat, protein, sugar or GE in five sampling events (5 animals) that were studied (repeated measures ANOVA, $p = 0.360$, 0.153, 0.361, 0.165, 0.223, respectively).

3.2 | Overall Lactation Trends

DM, fat, protein, sugar and GE exhibited marked changes in concentration over the course of lactation (Figures 2A,B, and 3A) but sample variance also changed, as evident in the increased

TABLE 2 | Milk constituents in primary versus supplemental periods of lactation[a].

Constituent[b]		Primary (0.04–0.57 y)			Supplemental (0.60–2.20 y)			Mann-Whitney (*T*) or *t*-test (*t*)
		Mean	**SEM**	***N***	**Mean**	**SEM**	***N***	
Water	%	**86.1**	0.40	32	**74.0**	1.38	39	$T = 1700$***
DM	%	**13.9**	0.40	32	**26.0**	1.38	39	$T = 604$***
Fat	%	**4.25**	0.332	32	**15.9**	1.24	38	$T = 562$***
Protein	%	**1.62**	0.076	32	**3.84**	0.297	40	$T = 635$***
Sugar	%	**7.84**	0.142	32	**6.31**	0.213	37	$t = T = 552$***
Total	%	99.8	0.24	32	99.6	0.24	35	$T = 1124$ NS, $p = 0.656$
GE	kcal/g	**0.792**	0.0320	32	**1.91**	0.131	35	$T = 564$***
Fat energy[c]	% GE	**46.6**	2.17	32	**72.7**	1.54	35	$t = T = 568$***
Protein energy[c]	% GE	12.3	0.54	32	11.7	0.74	35	$t = -0.575$ NS, $p = 0.567$
Sugar energy[c]	% GE	**41.1**	1.86	32	**15.6**	1.34	35	$T = 1627$***
Ca	%	0.083	0.0061	27	0.125	0.0181	12	$T = 298$ NS, $p = 0.078$
P	%	**0.026**	0.0017	23	**0.050**	0.0069	8	$T = 188$**
Mg	%	0.012	0.0010	27	0.016	0.0018	12	$t = 1.766$ NS, $p = 0.086$
Ca:P	g/g	2.81	0.165	22	2.36	0.426	7	$t = 1.184$ NS, $p = 0.247$
Zn	ppm	**3.32**	0.217	23	**11.9**	2.17	8	$T = 209$***
Na[d]	mM/kg H_2O	**18.1**	1.21	27	**28.5**	3.96	12	$T = 313$*
K[d]	mM/kg H_2O	4.01	0.337	27	5.42	0.749	12	$T = 289$ NS, $p = 0.136$
Cl[d]	mM/kg H_2O	**16.2**	1.33	17	**28.2**	5.03	4	$t = 3.342$**

[a]Statistically significant results are highlighted in bold font; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
[b]Concentrations expressed on a wet weight basis unless otherwise indicated.
[c]Expressed as a percentage of total calculated gross energy.
[d]Expressed relative to milk water content.

scatter of points after ~0.6 y, complicating the interpretation of trends with infant age. Testing of statistical significance of regression models required many variables to be ln transformed to conform to ANOVA requirements of normality and constant variance (see Supplemental Table S1). Nonetheless, DM, fat, protein, GE and FatEn% increased with infant age, while sugar and SugEn% decreased; ProtEn% did not change (Supplemental Table S1). The mineral elements of Ca and P (Figure 4) and Mg and Zn (Figure 5) all increased with infant age, although only some models (P, Zn) had a significant quadratic and/or cubed term (Supplemental Table S1). The ratio of Ca:P in Figure 4 averaged 2.70 and did not change with infant age (Supplemental Table S1). When expressed as molal concentrations (mM/kg water) the milk electrolytes, Na and Cl increased with infant age, but K did not (Figure 6; see Supplemental Table S1).

3.3 | Comparison of Two Periods

Our data encompass the primary period of lactation up to about 7 mo, when most infants continue to nurse even as they begin to consume solid foods, and the supplemental period when offspring are largely feeding themselves but continue to receive milk (7 mo to 1.5 y or longer; see Figure 2).

Given the demonstrated effect of iAge, and loss of milk water content it is not surprising that the mean values for the concentrations of milk DM, fat, protein, GE and FatEn% were significantly higher in the supplemental period (≥ 0.58 y) than during primary (< 0.58 y) lactation (Table 2), whereas Sugar and SugarEn% were significantly lower. There was no difference in ProtEn% between the two periods (Table 2). Among mineral elements, the mean values for P and Zn were significantly higher in the supplemental period, but there were no statistical differences between the periods in Ca or Mg (Table 2). Among the electrolytes, Na and Cl were significantly higher in the supplemental period, but K did not differ between the two periods (Table 2).

3.4 | Compositional Trends Within Each Period

We also examined the relationship of milk variables to iAge within each period, so that our results on wild toque macaque milk could be compared to previous studies of the milk of cercopithecines which only encompass part or most of the primary lactation period (see Discussion, below). Within each period of lactation, the regression residuals of most milk constituents passed normality and constant variance. Exceptions in primary lactation involved two nonsignificant regressions (K and protein) that failed normality. Within the supplementary period all values passed normality, but Zn and Cl (with few df) and sugar failed constant variance (Table 3).
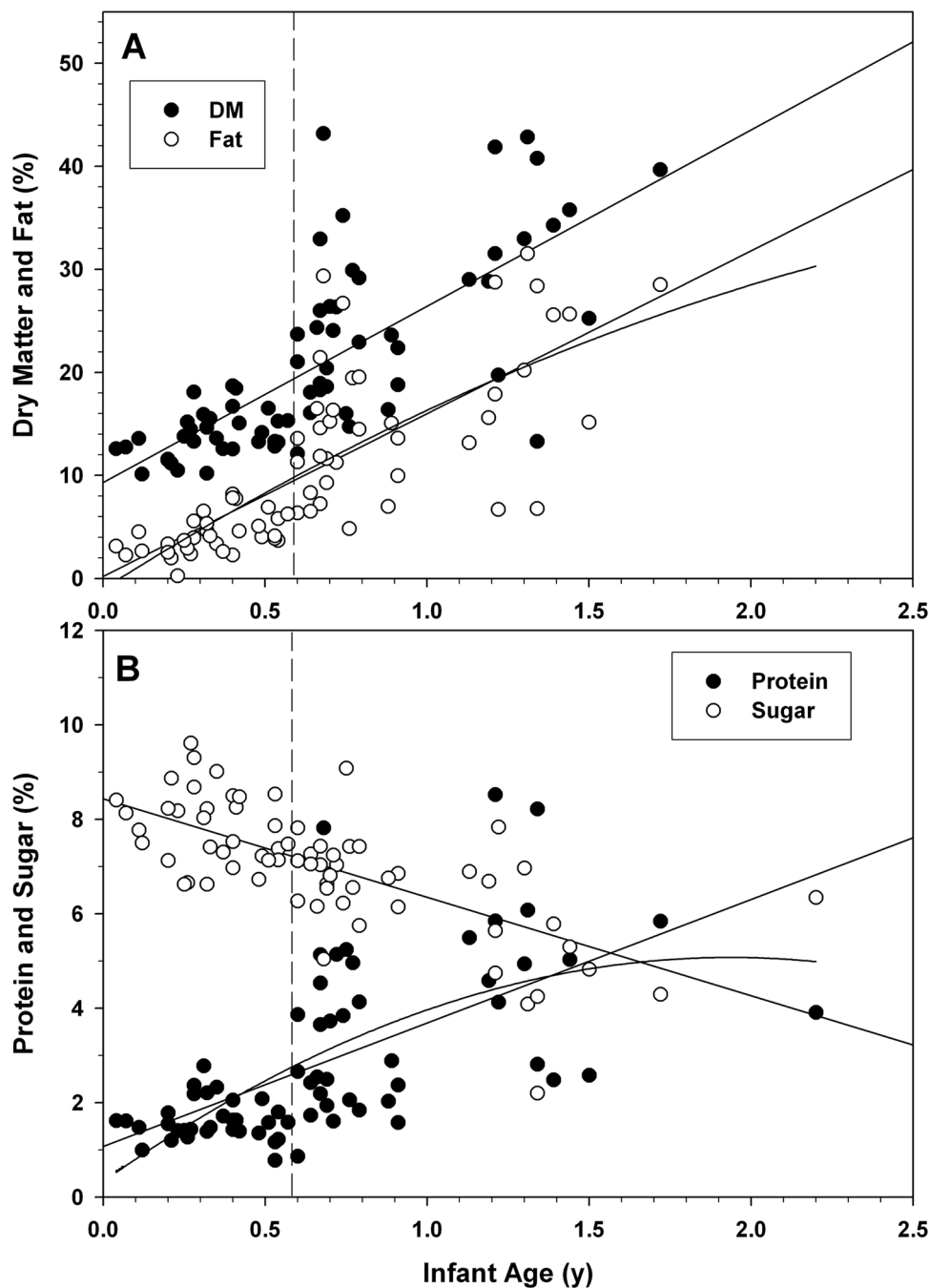
FIGURE 2 | Changes in the composition of toque macaque milk in relation to infant age encompassing the primary (< 0.583 y or 7 mo) and supplementary (≥ 0.583 y, or 7 mo) phases of lactation in terms of the proportions of dry matter and fat (A), as well as protein and sugar (B).

During primary lactation, there was a significant linear decrease in water and increase in DM, fat, GE, and FatEn% in relation to iAge, but ProtEn% and SugEn% decreased (Table 3). There were no significant changes in protein, sugar, or any of the mineral elements or electrolytes (Table 3).

As the supplemental period has not previously been examined in any cercopithecid, the changes during this period were of particular interest (Table 3). During supplemental lactation, water continued to decrease linearly whereas DM, fat, GE and FatEn% continued to increase in linear fashion, but in this period protein, P and Zn increased with iAge. Both Sugar and

SugEn% declined with infant age, while Ca, Mg and the three electrolytes (Na, K, Cl) did not exhibit significant change.

3.5 | Mineral Elements and Electrolytes in Toque Macaque Milk

There were no significant effects of infant age on any mineral element (Ca, P, Mg, Zn) or electrolyte (Na, K, Cl) during the primary lactation period in *M. sinica* (Table 3, Figures 4–6) and therefore the average values for these constituents were compared to prior studies of cercopithecine milks (Table 4).

TABLE 3 | Regression analysis of milk constituents on infant age during primary and supplemental lactation periods[a].

		Primary lactation period				Supplemental lactation period			
		Regression[b]				Regression[b]			
		a	b	DF	F-statistic	a	b	DF	F-statistic
Water	%	**88.05**	**−5.955**	31	5.22*	**86.52**	**−13.56**	38	11.71**
DM	%	**11.9**	**5.955**	31	5.22*	**13.48**	**13.56**	38	11.71**
Fat	%	**2.19**	**6.134**	31	8.77**	**5.57**	**11.13**	37	9.701**
Protein	%	1.66	−0.128	31	0.057 NS, $p = 0.813$	**2.20**	**1.713**	39	4.831*
Sugar	%	8.26	−1.26	31	1.66 NS, $p = 0.208$	**8.04**	**−1.769**	36	12.60***
GE	kcal/g	**0.624**	**0.502**	31	5.88*	**0.820**	**1.151**	34	9.865**
FatEn[c]	% GE	**32.3**	**42.58**	31	10.28**	**62.2**	**11.05**	34	5.934*
ProtEn[c]	% GE	**15.2**	**−8.651**	31	6.12*	9.37	2.505	34	1.167 NS, $p = 0.288$
SugEn[c]	% GE	**52.5**	**−33.93**	31	8.54**	**28.4**	**−13.56**	34	14.43***
Ca	%	0.0765	0.0185	26	0.19 NS, $p = 0.671$	0.0486	0.0615	11	0.531 NS, $p = 0.483$
P	%	0.0236	0.00901	22	0.58 NS, $p = 0.454$	**−0.0259**	**0.0704**	7	55.010***
Mg	%	0.0118	0.00159	26	0.05 NS, $p = 0.825$	0.00317	0.0102	11	1.576 NS, $p = 0.238$
Zn	ppm	3.64	−1.022	22	0.44 NS, $p = 0.514$	**−6.72**	**17.31**	7	7.215*
Na[d]	mM/kg H_2O	16.3	5.187	26	0.37 NS, $p = 0.548$	−0.922	23.76	11	1.863 NS, $p = 0.202$
K[d]	mM/kg H_2O	4.01	−0.0079	26	0.00 NS, $p = 0.997$	1.78	2.943	11	0.720 NS, $p = 0.416$
Cl[d]	mM/kg H_2O	10.8	16.24	16	3.23 NS, $p = 0.093$	0.612	26.18	3	3.204 NS, $p = 0.215$

[a]Concentrations expressed on a wet weight basis unless otherwise indicated. Statistically significant results are highlighted in bold font.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$
[b]Regression model: $Y = a + bx$, where x = infant age.
[c]Expressed as a percentage of total gross energy.
[d]Expressed relative to milk water content.

TABLE 4 | Mineral Elements and Electrolytes in Cercopithecine Milks[a].

	Infant Age (days)	N	Ca %	P %	Ca:P	Mg %	Zn ppm	--------mM/kg H_2O-------			Ref[b]
								Na	K	Cl	
Macaca fascicularis	44–119	8	0.039	0.015	2.55	0.0034		47.7	6.2		1
M. fuscata	28–105	9						9.2	5.8	19	2
	119–196	9						14.7	4.8	33	2
M. mulatta	16–35	43	0.042			0.0033	1.84	4.6	7.8		3
	36–48	18	0.039			0.0031	1.86	4.2	8.4		3
	90–120	104	0.051	0.030	1.70						4
	15–92	6	0.054	0.031							5
M. radiata	57–133	10	0.047			0.0050		8.0	11.4	32	6
	151–216	10	0.051			0.0057		12.3	10.9	46	6
M. nemestrina	70–98	5	0.060	0.032	1.80		3.30				7
M. sinica	15–208	17–27	0.083	0.026	2.81	0.0120	3.32	18.1	4.0	16	8
	219–423	4–12	0.125	0.050	2.26	0.0160	11.9	28.5	5.4	28	8

[a]See Table I for common names.
[b]References: 1. Nishikawa et al. 1976; 2. Ôta et al. 1991; 3. Lönnerdal et al. 1984a; 4. Hinde et al. 2013; 5. Glick et al. 2021; 6. Laudenslager et al. 2010; 7. Glick and Power 2023; 8. This study.

4 | Discussion

4.1 | Chemical Analysis of Primate Milks

It is well known in sampling of human milk and the milk of some dairy animals that fat content may rise during the course of mammary evacuation (Daly et al. 1993; Oftedal 1984; Oftedal and Iverson 1995) but whether this occurs in cercopithecines is not known. We did not find any significant increase in fat content of sequential samples of toque macaque milk obtained during milking, but the sample size was modest ($n = 5$ females). Given that milk fat content may reflect the amount of milk

remaining in mammary glands at the time of milk collection (Daly et al. 1993) it is possible that the variability we observed in fat content of toque macaque milk was influenced by nursing events before troop capture, but this is unlikely to have affected mean values of milk fat.

Milk has evolved as a secretory fluid with unique properties, such as the dispersion of protein in large calcium-and phosphate-stabilized casein micelles, the packaging of lipids into membrane-bound milk fat globules, and the presence of milk-specific sugars such as lactose, sialyllactose and larger milk oligosaccharides (Goto et al. 2010; Holt et al. 2013; Nishikawa et al. 1976; Oftedal 2013). Analytical methods need to consider these properties, as well as specific challenges associated with analysis of high fat and high oligosaccharide milks, or erroneous results may be produced (Oftedal et al. 2014). The assays reported herein are either reference methods for milk (oven-drying, Roese-Gottlieb, Kjeldahl, phenol-sulfuric acid) or were validated against them (Oftedal et al. 2014); assays for mineral elements were validated against standard milk reference material. We ascertained that the method of energy calculation matched energy content determined by bomb calorimetry, and noted that the sum totals of assayed water, fat, protein and sugar averaged 99.8% and 99.6% in the primary and supplemental periods, respectively (Table 2). The remaining 0.2%–0.4% is mostly attributable to aggregate mineral residue or ash, estimated as 0.2%–0.4% in cercopithecine milks (Buss 1968; Buss and Cooper 1970; Nishikawa et al. 1976).

Some of the discrepancies in comparing prior studies of cercopithecines to the current findings (Table 1) may reflect analytical bias. For example, in analyzing milk fat, the Folch method used for *Chlorocebus pygerythrus* and *M. fuscata* milks may under-report fat content, at least in high fat milks, while the creamatocrit method used for *Papio* spp. and *M. radiata* milks requires careful calibration across the full range of measured fat concentration (Oftedal et al. 2014). The Lowry method used to measure protein in *M. fuscata* milk is subject to such large error as not to be suitable for milk analysis (Keller and Neville 1986; Oftedal et al. 2014). The enzymatic methods used for sugar determination in *M. fuscata* and *M. radiata* milks entirely omit oligosaccharides, while reducing sugar methods used for *Cercopithecus talapoin* and *Papio* spp. milks will underestimate them (Eisert et al. 2013; Oftedal and Iverson 1995; Oftedal et al. 2014). This is problematic in that about 14%–20% of the total sugar in cercopithecid milks is due to oligosaccharides, including 3-fucosyl lactose, Lacto-N-fucopentaose III [Gal(β1-4)[Fuc(α1-3)] GlcNAc(β1-3)Gal(β1-4)Glc] and 3′-N-Acetyl-sialyllactose, all of which are found in toque macaque milk (Goto et al. 2010). Errors in assay of any constituent will also impact the calculated energy content, and the proportions of energy derived from different milk constituents (Table 1). Our finding that calculated gross energy in toque macaque milk matched measured energy content implies that our assays were reasonably accurate.

4.2 | Toque Macaque Milk Compared to Milks of Other Cercopithecines During Primary Lactation

All prior studies of cercopithecine milks have been restricted to the primary lactation period (< 7 mo iAge; Table 1), except a few samples of baboon milk included in the final (36–279 d)

milk sample group by Buss (1968). We therefore compare our results for primary lactation (Tables 2, 3) to prior studies on other cercopithecine species (Table 1). To facilitate these comparisons, we also present our toque macaque data for the first four mo (15–120 d) separately in Table 1. Compared to other mammals (Oftedal and Iverson 1995), cercopithecine milks in early to mid-lactation (i.e. the first 4 months, Table 1) are relatively dilute (12%–16% dry matter), modest in fat (2.5%–5.2%) and protein (1.5%–2.2%) and high in sugar (7.3%–8.5%; lower values obtained by difference or by enzymatic lactose method excluded). In captive *M. nemestrina* the unusually high body condition among dams, presumably from adipose tissue (e.g., Dittus 2013), contributed to comparatively high gross energy (102.0 kcal/100 g) mostly from fat (59.6%) (Glick and Power 2023). Cercopithecine milks contain about 13%–14% of energy as protein (range 11%–20%, Table 1); in earlier compilations average values of 12.5% and 14.0% of energy in cercopithecine milk were reported by Power et al. (2002) and Hinde and Milligan (2011). At this lactation stage toque macaque milk resembles the proximate milk composition of other cercopithecines, as far as differences in analytical methodology allow conclusions to be drawn.

It is important to emphasize that the data in the present study are cross-sectional and involve a wild population; although three females were milked more than once, these were during separate lactations involving different infants in different years. All previous studies on cercopithecines have been conducted in breeding colonies and usually entail repeated sampling of the same individuals. We found a small but significant linear increase in DM, fat and GE during primary lactation in *M. sinica* (Table 3). Since the slopes of DM (6.0%/y) and fat (6.1%/y) are virtually identical, and the only energetic constituent that increased was fat energy (Table 3), we attribute the increase in all three constituents to the change in fat content; neither protein nor lactose changed significantly. Ôta et al. (1991) reported a significant increase in DM and fat content of *M. fuscata* milk from 1 to 1.5 mo to 6–6.5 mo, although the increase is much larger than what we observed before 7 mo. In captive *M. nemestrina* milk, too, Glick and Power (2023) noted significant increases in fat and DM and therefore in GE between 10 and 14 weeks postpartum. Fat content exhibits an apparent rise over the primary lactation period (Table 1) in the milks of *M. fuscata* (Ôta et al. 1991), *M. mulatta* (Hinde et al. 2009; contra Lönnerdal et al. 1984a), *M. radiata* (Laudenslager et al. 2010) and *M. sinica* (our study), but not in *Papio* spp. (Buss 1968; Roberts et al. 1985), but constraints on sample size, experimental design and data presentation do not allow statistical evaluation in all studies.

Although we did not find a rise in protein content during the primary lactation period in *M. sinica*, a small but highly significant rise in milk protein is observed from 1 to 7 mo in provisioned *M. mulatta* (Hinde et al. 2009); the repeated measures design allows partitioning of significant interindividual variation. Glick and Power (2023) report a slight (1.7% to 1.9%) but nonsignificant increase in the contribution of protein to GE in the comparative short period from 10 to 14 weeks postpartum among provisioned *M. nemestrina*. Ôta et al. (1991) also report both interindividual difference and a large rise in milk protein content in *M. fuscata* as assayed by the Lowry method, whereas

Lönnerdal et al. (1984a) did not find a significant change in Lowry protein content from the 0.25 to 1.5 mo in *M. mulatta*. However, Lowry data cannot be considered reliable as they are influenced by a multitude of factors, including changes in types of protein, amino acid composition of proteins and non-protein interfering compounds, that generate spurious results (Keller and Neville 1986; Oftedal et al. 2014). It is not possible to separate analytical errors from biological effects when an unvalidated and potentially biased method, such as the Lowry method, is utilized (Oftedal et al. 2014). Even with total nitrogen (N) assays, such as the Kjeldahl, Nesslerization and Dumas (CHN) methods (Oftedal et al. 2014), care must be taken in interpretation as non-protein nitrogen sources, such as urea, glutamine, creatinine, nucleic acids and other N-containing metabolites, account for about 20%–25% of total nitrogen in human milk (Atkinson and Lönnerdal 1995), and 11% in *M. fascicularis* milk (Nishikawa et al. 1976). Not only may these constituents be differently affected by exogenous factors such as diet and metabolic stress, but they also change with lactation stage, at least in human milk (Atkinson and Lönnerdal 1995). Our species-specific estimates of milk protein and ProtEn% in Table 1 are overestimates in that they assume all N is protein N.

In *M. sinica*, milk sugar content did not change significantly over the primary lactation period (Table 3). A small but significant decline in milk sugar from 7.6% at 1 mo to 7.5% at 7 mo was observed in provisioned *M. mulatta* (Hinde et al. 2009) but is unlikely to have biological importance. Similarly, among provisioned *M. nemestrina* there was a slight but significant decrease in milk sugar content from 7.7% to 7.4% between 10 and 14 weeks (Glick and Power 2023). Comparisons of our data to other studies are complicated by the preponderance in these studies of analytical methods that do not accurately report milk oligosaccharide content and thus underestimate total sugar content of cercopithecine milks. The phenol-sulfuric acid method employed herein and by Hinde et al. (2009) is most inclusive among available sugar methods but still omits the N-acetylated saccharides present as components of oligosaccharides (Oftedal et al. 2014).

Overall, the pattern of total milk energy among well-nourished human-managed cercopithecines is similar to that in wild toque macaques during the period of primary lactation (< 7 mo) insofar as with increasing infant age most energy was derived progressively more from fat, less from sugar, while the energy contribution of protein remained relatively constant.

4.3 | Mineral Elements for Growth in Milks

Ca and P are present in milk as components of casein micelles but are also present in solution in ionic form in association with other solutes (Atkinson et al. 1995; Holt et al. 2013; Neville et al. 1994), as we determined Ca and P on milk digests they encompass all Ca- and P-containing constituents and are reported as a percentage of milk mass (Table 4). Milk Ca and P are both important as elemental sources for deposition of bone mineral during infant postnatal growth (Hinde et al. 2013; Prentice 2003). In human milk, both Ca and P decline slowly from about 0.026% Ca and 0.016% P in the 1st mo to 0.019% Ca

and 0.014% P at 12 mo (Atkinson et al. 1995). Our average value of 0.083% Ca in *M. sinica* milk is somewhat higher than values (range 0.039%–0.054%) reported for *M. fascicularis*, *M. mulatta*, *M. radiata*, and *M. nemestrina* whereas the average *P* value of 0.026% is higher than that of *M. fascicularis* but slightly lower than that of *M. mulatta* and *M. nemestrina* (Table 4). As a consequence, the calculated Ca:P ratio for toque macaque milk is somewhat higher than that for *M. fascicularis*, *M. mulatta* or *M. nemestrina*. The significance of these minor differences are speculative given that samples involve different species, ages, nutritional environments, dams of varying body condition, and analytical methods. However, all primate milks are relatively low in Ca and P compared to the milks of other mammals, which in species such as rabbits and bears may reach > 0.4% Ca and > 0.27% P (Jenness 1979; Oftedal 1993).

Mg is an essential nutrient for bone development and growth (Miller et al. 1965), while Zn as a trace element has been considered a potentially limiting nutrient for human breast-fed infants in late lactation (Krebs and Hambidge 1986). The 0.012% Mg and 3.32 ppm Zn levels in *M. sinica* milk were somewhat higher than previous reports for other macaques, except for the milk of M. nemestrina where Zn at 3.30 ppm was equally as high as in *M. sinica* (Table 4). Lönnerdal et al. (1984b) observed a significant decline in milk Zn in the 1st mo postpartum in *M. mulatta*, a period when we had few samples (Figure 5B), but the quadratic equation that explained all Zn data (both primary and supplemental periods; supplemental table S1) suggests that an initial decline may have occurred (Figure 5B). Marked decline in milk Zn in early lactation is also observed in the first months of human lactation (Casey et al. 1995; Krebs et al. 1995).

4.4 | Electrolytes and Milk Secretion

The electrolyte composition of milk, expressed per kg water, represents the secretory activity of the mammary gland. Milk electrolytes are not actively pumped across the apical membrane facing the lumen in secretory alveoli, and thus milk electrolytes reflect both equilibria with intracellular electrolytes in the mammary secretory cells and passive movement of extracellular electrolytes between the secretory cells into milk via a paracellular pathway (Shennan and Peaker 2000). In particular, a rise in milk Na and Cl and a fall in milk K is usually taken to mean an increased paracellular flux, as occurs in the rabbit and other taxa in late lactation but may also occur during breakdown of the mammary secretory epithelium during mastitis and mammary involution (Atkinson et al. 1995; Peaker and Taylor 1975; Shennan and Peaker 2000). The fact that there were no significant changes in Na, K or Cl during primary lactation in *M. sinica* (Table 3) is consistent with the hypothesis that toque macaques had not begun to "dry up" or cease active milk production during the first 7 mo after birth. Our average Na concentration, (18.1 mM/kg H_2O; Table 2), was within the range of values previously reported for macaques (4–48 mM/kg H_2O), although why there should be such variation among samples (Figure 6A), studies and species (Table 4) remains unclear. One concern is that repeated use of large oxytocin doses may disrupt the mammary epithelium and lead to

"leakage" of Na into milk (e.g., Linzell et al. 1975). Ôta et al. (1991) considered the progressive rise in Na from 5 mM/kg H_2O at 1–1.5 mo to 19 mM/kg H_2O at 6–6.5 mo in *M. fuscata* as likely due to repeated oxytocin administration during sequential milkings of individual monkeys. Our study involved no more than one milking per lactation per toque macaque and no significant change in Na during the primary lactation period (Table 3). Another possible error could arise from sweat- or environmentally derived sodium that coats nipples and thereby contaminates milk during collection (Oftedal et al. 1987). We rinsed the nipples with distilled water before milk collection but this was apparently not done in the *M. fascicularis* study in which the highest Na levels are reported (Table 4).

During the primary lactation period *M. sinica* milk contained 4.0 mMol K/kg H_2O, which is somewhat less than K concentrations previously reported for other *Macaca* spp (4.8–11.4 mMol/kg H_2O; Table 4). During the first 7 mo we observed no effect of infant age on milk K in *M. sinica*; this is consistent with data on *M. mulatta* (first 1.5 mo [Lönnerdal et al. 1984a] and *M. radiata* (2–7 mo [Laudenslager et al. 2010]), but Ôta et al. (1991) observed a significant decline in K per kg H_2O from 1 mo to 6–6.5 mo in *M. fuscata*. The Cl content of *M. sinica* milk was similar to, but slightly less than, that of other studied *Macaca* species (Table 4) and did not change during the primary lactation period, in contrast to the progressive rise in milk Cl from 1 to 6 mo in *M. fuscata* (Ôta et al. 1991).

4.5 | Changes in Milk Composition During the Supplemental Lactation Period and Its Biological Implications

An underlying premise of this study is that lactation can be subdivided into the primary lactation period – the period for which milk has previously been studied in cercopithecines – and a supplemental period during which nursing time is much reduced and offspring feed predominantly on solid foods. We hypothesized that the milk secreted in the secondary period has evolved to meet the needs of older offspring for supplemental nutrients (over and above what is obtained from natural foods) and thus represents a transitional phase between nutritional dependence on milk and independent feeding. Our selection of 7 mo as the partition between the two periods relates to a multitude of changes in maternal and infant behavior that occur at about this infant age in *M. sinica* and other cercopithecines (Dittus and Baker 2024).

Two features were characteristic of the supplemental period: 1. An increased variance of the data by comparison to the primary period (see Figures 2–6); 2. Lower water and therefore higher concentrations of many constituents were evident - including DM, fat, protein, energy, fatEn%, P, Zn, Na, and Cl; along with water, only sugar and sugEn% declined (Table 2). Thus, the supplemental period of lactation is clearly not just an extension of the primary lactation period. If the supplemental period is considered separately, the densities of DM, fat, protein, energy, P, and Zn continued to rise in linear fashion as infants got older (Table 3). Some or all of this increase in concentrations on wet weight basis is attributed to lower water content associated with a concomitant decline in sugar synthesis (Tables 3 and 4).

A major behavioral difference in the supplemental period is the decline in time spent nursing by *M. sinica* infants after 7 mo (Dittus and Baker 2024). In *M. fuscata*, Tanaka (1992) noted not only a decline in time spent nursing but also a marked decline after 6 mo in the duration of bouts that entail constant rates of sucking. By analogy to human sucking behavior Tanaka (1992) assumed that bouts so defined represent milk consumption, and thus termed his measure a "rate of milk transfer" even though milk transfer (as discussed by Oftedal [1984] and Riek [2008] was not measured). An increase in milk nutrient concentration in the supplemental period counteracts the diminishment in nutrient intakes that occurs from declining milk yield during extended lactation (Oftedal 1985). An increase in milk nutrient concentration also permits greater nutrient storage in mammary glands between nursing bouts, a factor that appears to have played a role in the evolution of energy and nutrient-dense milks in prosimian primates (Tilden and Oftedal 1997), bats (Kunz et al. 1995) and otariid seals (Oftedal et al. 1987). In other words, a lactating female producing more concentrated milk can afford to nurse her offspring less frequently, which may be an important part of the increasing independence and social development of the young. In particular, a decrease in nursing time during routine group daytime resting sessions frees juveniles not only to extend their foraging time, but also to forage in a less competitive context while older dominant group members rest (Figure 12 in Dittus 1977a).

The primary mechanism by which milk energy density, per unit wet weight, is increased in *M. sinica* is via increased fat concentration, which more than triples between the primary and supplemental periods, leading to a substantive increase in milk energy concentration (from 0.792 to 1.91 kcal/g) and fatEn% (from 46.6% to 72.7%; Table 2). In contrast to the primary period, in which milk composition in *M. sinica* resembles that of other cercopithecines, the mammary secretions of *M. sinica* in the supplemental period are highly divergent from previous primate data. Hinde and Milligan (2011) summarized milk composition data for 37 primate species, and all fell within the range of 0.2%–8.0% fat concentration except one prosimian (*Galago moholi*, 12.7% fat [Tilden and Oftedal 1997]). In the supplemental period, 32 of 38 samples of *M. sinica* milk were greater than 8.0% fat, and 10 of 38 samples were greater than 20% fat (Figure 2). Such high fat levels resemble those of rodent and terrestrial carnivore milks more than primate milks (Hood et al. 2014; Oftedal and Iverson 1995; Senda et al. 2010; Taufik et al. 2013; Uemura et al. 2009). The increase in fat density of *M. sinica* milk indicates that the rate of synthesis and secretion of milk fat globules outpaces that of secretion of the aqueous phase, which depends on the exocytosis of secretory vesicles into the mammary lumen (Oftedal 2013).

According to accepted models of milk secretion, lactose synthesis drives aqueous phase secretion via osmotic effects that draw water into the secretory vesicles (Shennan and Peaker 2000). Although sugar concentration in *M. sinica* was significantly lower in supplemental than primary milk (Table 2) it nevertheless remained high (6.31% on average) declined only gradually with infant age (slope of −1.77% per year, or −0.15% per month), and was within the range (5.1% to 8.5%) of equivalent values among captive cercopithecines (Table 1). This suggests that aqueous phase synthesis, and thus milk yield,
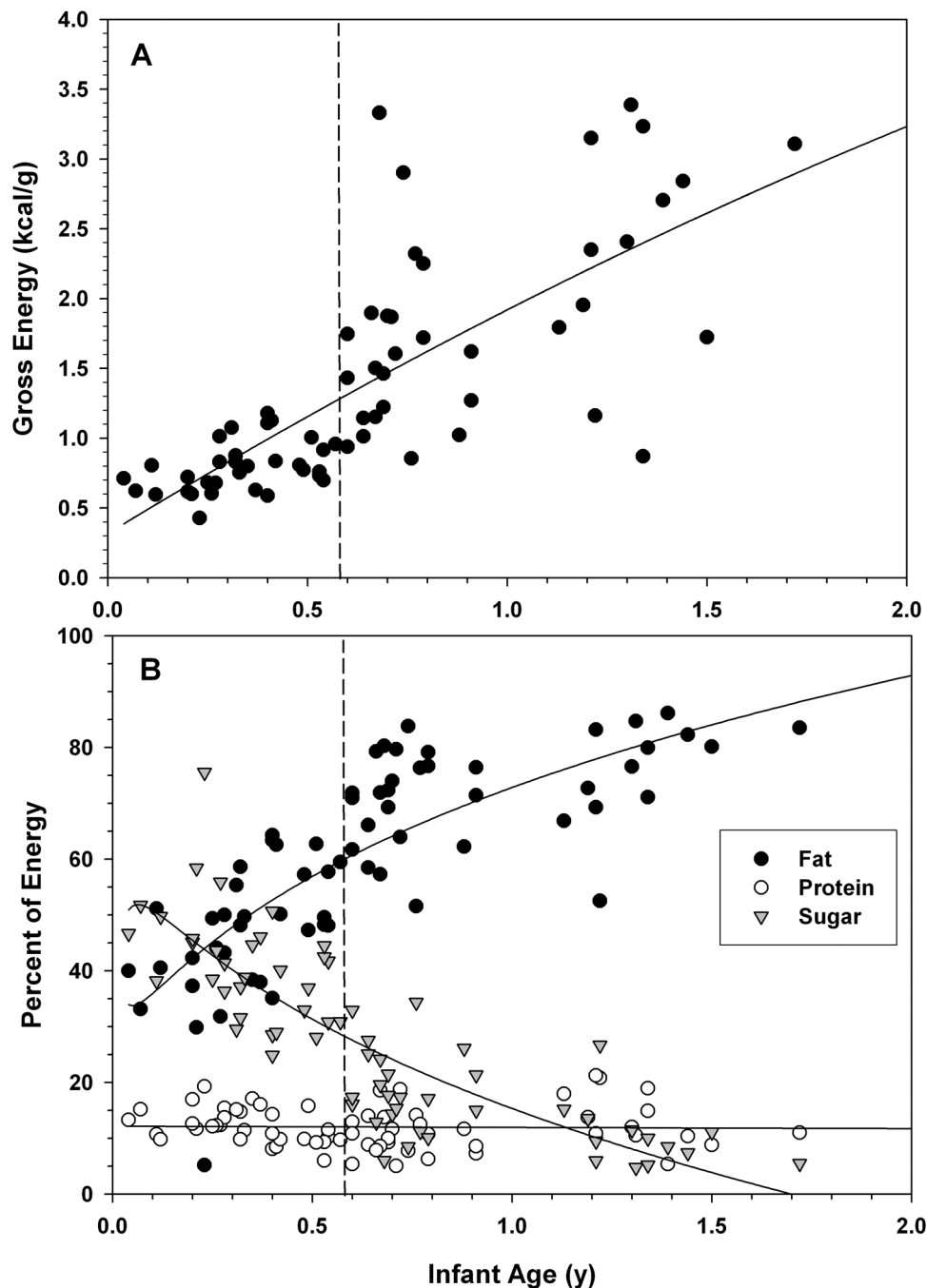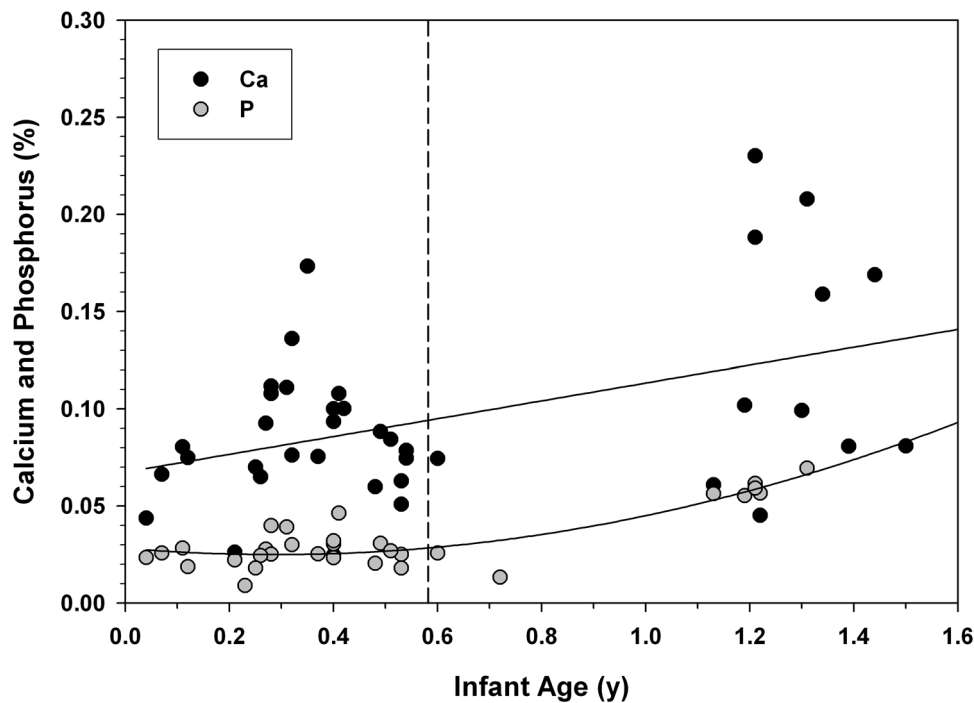
FIGURE 3 | Changes in the composition of toque macaque milk in relation to infant age encompassing the primary (< 0.583 y or 7 mo) and supplementary (≥ 0.583 y, or 7 mo) phases of lactation in terms of gross energy (A) and the percentages of energy contributed by each of the constituents: fat, protein, and sugar (B).

remain substantial in *M sinica* during the supplemental period, although undoubtedly declining.

In some mammals, milk Na and Cl rise in late lactation, indicative of increased passage of these electrolytes into milk via paracellular pathways in the mammary gland (Peaker and Taylor 1975; Shennan and Peaker 2000). This appears to occur to some extent in *M. sinica*, in that both of these electrolytes are higher in the supplemental period, but there were no significant changes in Na or Cl within this period (Table 3), perhaps associated with small sample numbers (Figure 6, Na, $n = 12$; Cl, $n = 4$). However, Na was negatively correlated to sugar

concentration in the supplemental period (Na, mMol/kg $H_2O = -6.7581$*sugar% + 68.36, $r^2 = 0.422$), a relationship that suggests that a decline in sugar synthesis and loss of mammary epithelial integrity may have occurred in some animals close to the end of lactation. A marked rise in milk Na and Cl, with a concurrent decrease in K and lactose, are hallmarks of mammary involution and/or mastitis (Atkinson et al. 1995). While some macaques with exceptionally high Na and Cl levels at or after 1.2 y iAge (Figure 6) may have been at the end of lactation, the sustained lactose and K levels during the supplemental period indicate that milk secretion proceeded normally for most animals.

FIGURE 4 | Changes in the composition of toque macaque milk in relation to infant age encompassing the primary (< 0.583 y or 7 mo) and supplementary (≥ 0.583 y, or 7 mo) phases of lactation in terms of the proportions of the minerals Calcium and Phosphorus.

During the supplemental period milk provides a potentially important source of nutrients – such as protein, Ca, P, Mg and Zn – required for tissue synthesis, including bone development (Prentice 2003), during body growth of macaque offspring. In a review of protein requirements of primates Oftedal (1991) concluded that older infant/young juvenile primates (5–9 mo iAge) require about 6%–10% of metabolizable energy to be provided by protein. Metabolizable energy of milk is somewhat lower than gross energy as it corrects for energy losses in feces and urine, but this is a relatively small correction as the energetic constituents of milk are nearly 100% digestible (Walker 1979). Thus, even though *M. sinica* milk in the supplemental period contains only 11.7% of energy as protein (Table 2), this is substantially higher than the estimated requirement (Oftedal 1991), and represents a high-quality protein source, containing both caseins and whey proteins of balanced amino acid composition (Oftedal 2013). Thus, *M. sinica* milk may be an important supplemental source of protein for macaque infants. The same is likely true for Ca, P, and Mg (Table 2), all of which are mobilized from maternal tissues to provide a milk nutrient source for growth of infant bone and other tissues (Hood et al. 2006; Prentice et al. 1994; Specker et al. 1994).

The dramatic rise in Zn in toque macaque milk during the supplemental period (Figure 5B) is of particular interest because it is at odds with the low Zn seen in late lactation in human milk (Krebs et al. 1995) despite an estimated doubling of the Zn requirement by human infants during the first 12 mo of life (Krebs and Hambidge 1986). The declining Zn content of human milk has been interpreted as a means of preserving maternal Zn body stores, but this apparently does not apply to toque macaques. In human infants, breastfeeding beyond 6 months of life entails risk of infant Zn deficiency, with adverse effects of growth and immune system function

(Hambidge and Krebs 2007). It may be that *M. sinica* infants in the weaning transition benefit from high supplemental zinc in milk because of low bioavailability of Zn in the diet onto which they are weaned, as for example due to phytates which interfere with Zn absorption from plant materials (Hurley 1985; Krebs 2000; Lönnerdal et al. 1984b). The essential role of zinc in promoting keranocytes in humans (Ogawa et al. 2018) suggests that it, too, may be important for hair development in toque macaques, where the adult pelage differs from that of newborn infants in the color, distribution, and structure of hair (Hill 1974), similar to that in many other mammals (Caro and Mallarino 2020). Research is clearly needed on the nutrient composition and secondary compounds in the foods eaten by weanling toque macaques.

The changes in milk composition show mean trajectories in relation to infant age and manifest considerable variation in the values of the different nutritional constituents that tend to increase in spread in late lactation (Table 3 and Figures 2–6). Infant age by itself, however, is not the only determinant. The females in this sample from 17 independent social groups were collected in five separate years and subject to different conditions such as social rank (priority of access to food and water), maternal age, rich versus natural diet, habitat change, and seasons. All these factors are known to influence interbirth intervals, adiposity, hydration, infection, infant growth and survival, and maternal body condition in toque macaques and other primates (Cheverud et al. 1992; Dittus 2004, 2013; Ekanayake et al. 2003). We predict that such inter-relationships also impact maternal balances of energy and nutrients that change with lactation duration and, in turn, underlie the observed variations not only in the duration of supplemental lactation (Dittus and Baker 2024) itself, but also in the concentrations of the different constituents in milk composition.
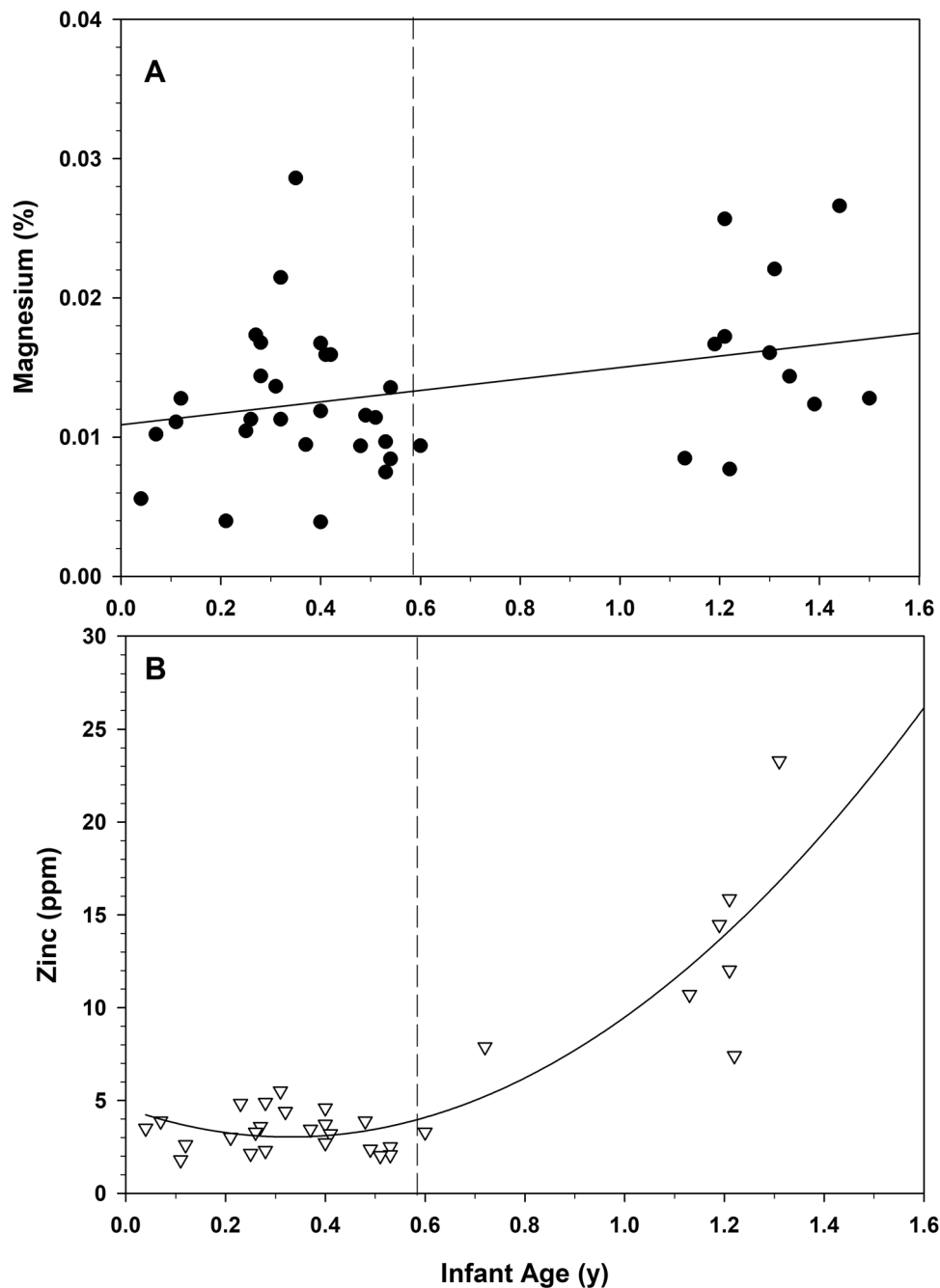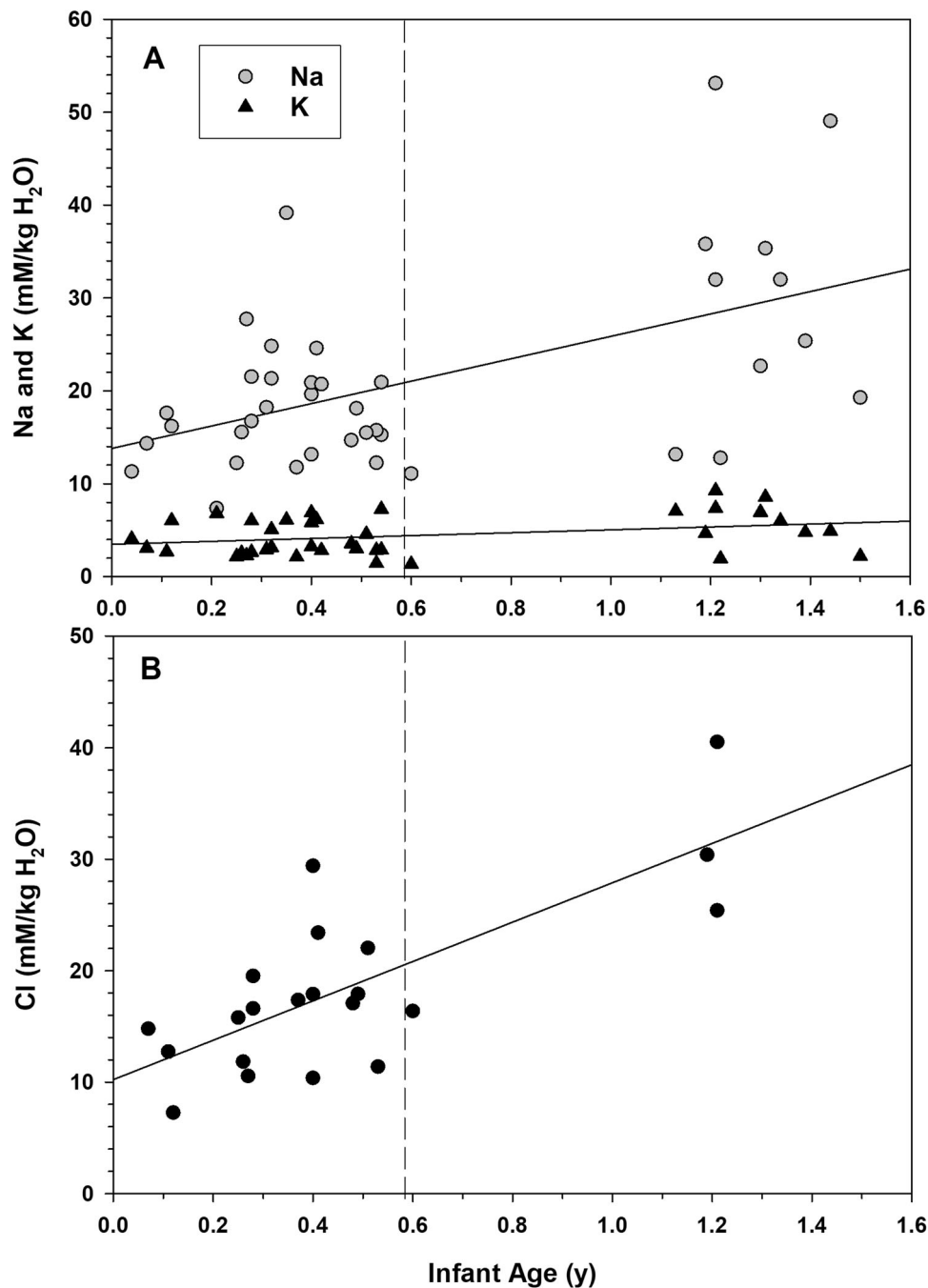
FIGURE 5 | Changes in the composition of toque macaque milk in relation to infant age encompassing the primary (< 0.583 y or 7 mo) and supplementary (≥ 0.583 y, or 7 mo) phases of lactation in terms of the percentage of Magnesium (A) and concentration of Zinc (B).

Bornbusch et al. (2024) reported that the macronutrient composition of milk among four species of captive cercopithecines was influenced more by species than environment. The natural environment, however, may have a greater role in the evolution that shaped species differences and that in the wild are subject to greater diversity and intensity of stressors than under human management.

5 | Conclusions

We set out to test the hypothesis that the composition of wild *M. sinica* milk differed from other cercopithecines, but our results support this hypothesis only in part. During the primary lactation period the major constituents in *M. sinica* milk were of similar concentrations as in other species, once lactation stage and differences in analytical methodology are considered. While some mineral elements were a bit higher (Ca, Mg, Zn) or lower (K, Cl) than previously published values, it is not clear if these discrepancies are due to species, analytical method, environmental contamination and/or small sample size. It has been previously observed (Oftedal and Iverson 1995) that milk composition rarely shows interspecific difference among congeneric species (e.g., in lemurs of the genus *Eulemur* [Tilden and Oftedal 1997]; in horses and asses of the genus *Equus* [Oftedal and Jenness 1988; Schryver et al. 1986], and in fruit bats of the genus *Pteropus* [Hood et al. 2001]) and the same appears to be true of species of *Macaca*.

FIGURE 6 | Changes in the composition of toque macaque milk in relation to infant age encompassing the primary (< 0.583 y or 7 mo) and supplementary (≥ 0.583 y, or 7 mo) phases of lactation in terms of the concentration of electrolytes sodium and potassium (A), as well as chloride (B).

Our results strongly support the hypothesis that the milk secreted in the supplemental period is markedly different from milk produced in the primary lactation period, and we suggest that this reflects the importance of such secretions as supplemental nutrient sources (of energy, protein, Ca, P, Mg, and Zn) for infant macaques. An alternative hypothesis was that milk collected from females after 7 mo simply represented residual amounts expressed from mammary glands undergoing involution due to cessation of active secretion. Our data do not support this hypothesis as milk sugar levels remained high (indicating active secretion of the aqueous phase of milk) and there were few pronounced or sudden jumps in Na or Cl that would indicate disruption of the secretory epithelium in the

mammary gland. While a couple of samples obtained at or after 1.2 y may have been at a terminal stage of lactation (based on reduced sugar and elevated Na), this was not true of most samples.

Blass and Teicher (1980) suggested that prolonged suckling behavior may be comforting and strengthening the mother-infant bond; our study shows that it is also nutritious.

The composition of toque macaque milk changes with infant age-related ability to self-feed nutrients from the environment. The natural diet of toque macaques comprises 77% fruit (Dittus 1977a; Hladik and Hladik 1972), which is generally

richer in sugars than other plant parts (Ko et al. 1998; Milton 1999), and access to free water are frequent requirements and seasonally limiting contested resources for toque macaques of all ages. For young infants, water and energy are critical nutrients that are supported during primary lactation by dilute milk, and mostly milk fat and sugar for energy (Figure 3B). Water and sugar become progressively more available to infants from the environment as they grow and transition to self-drinking and feeding fruit. After the initial weaning pulse at 7 months, when mothers resume reproduction, the push to infant independence is accentuated, water and sugar are reduced in supplemental milk, yet mothers continue to support their transitioning infants with many nutrients (including fat, protein, energy, P, and Zn) suggesting that these nutrients are less accessible from the environment than water and sugar. Among rapidly developing offspring with increasing nutrient needs, a shift to obtain water and sugar from the environment, rather than from milk, may lessen the maternal burden and prevent maternal depletion (Dittus and Baker 2024). This shift, albeit, while safeguarding maternal fitness deflects the risk of mortality to offspring that during drought, for example, die at greater rates than mothers in toque macaques (Table 1 in Dittus 1977a), as well as in *Lemur catta* (Gould et al. 1999) and *Cebus capucinus* (Campos et al. 2020). Such a transfer of risk is in keeping with parent-offspring conflict expectation (Trivers 1974; Williams 1966).

Data Availability Statement

Line 208. The methods applied, the metadata, and raw data content files relevant to this publication have been reposited for public access (Dittus et al. 2025).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.
Supplementary Table S1. Postable summary.