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RESEARCH ARTICLE

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Immunoglobulin A and nutrients in milk from great apes throughout lactation

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Differences in macronutrients between human and ape milks appear relatively small, but variation in other components such as immunoglobulins (Ig) may be greater. This study characterized the macronutrient and secretory (sIgA) profiles in milk from gorillas and orangutans throughout lactation. Fifty-three milk samples from four gorillas and three orangutans were collected throughout 48 and 22 months postpartum (MPP), respectively. Samples were grouped in five stages of lactation (0 to 6 months, more than 6 months to 12 months, more than 12 months to 18 months, more than 18 months to 36 months, and more than 36 months to 48 months). Data were analyzed as a complete randomized design. Concentration of sIgA did not change due to species or its interaction with MPP. Crude protein, regardless of MPP, was greater for gorillas compared with orangutans (1.27 vs. 0.85%). Fat, sugar, and gross energy were affected by the interaction of species × MPP. For gorilla milk, concentrations of sIgA were 43 mg/L at 6 MPP increasing to 79 mg/L at 48 MPP. Protein was highest at 48 MPP. Sugar was lowest at 48 MPP. Values for fat and gross energy were the highest 36 MPP. For orangutan milk, concentrations of sIgA were highest at 6 MPP. Sugar decreased with MPP. Protein, dry matter, or fat were unaffected by MPP. Gross energy content was steady during the first 18 MPP but it tended to decrease by 36 MPP. The results indicate that macronutrients are similar between human, published data, and great ape milk, though gorilla milk has higher protein and human milk higher fat (published data). Concentrations of sIgA in ape milk were about 10-fold lower than human values from the literature. Differences between human and ape milk may lie more in bioactive/immune molecules than nutrients.

KEYWORDS
gorilla, Immunoglobin A, milk, nutrients, orangutan

RESEARCH HIGHLIGHTS

- Milk macronutrients from great apes differed throughout lactation.
- Milk macronutrients but not IgA from non-human great apes and humans were quite similar.
- Milk protein was greater in Gorilla compared with Orangutan.

1 | INTRODUCTION

Milk is a complex biological fluid composed of the nutrients, hormones, and immunological constituents required for neonatal growth, development and health (Hinde and Milligan, 2011; Skibiel and Hood, 2015). Across different mammals, large variations in proximate constituents of milk (protein, sugar, and fat) can exist (Skibiel and Hood, 2015). However, anthropoid primate milks assayed to date, including human milk, are relatively similar in having a high sugar content and low-to-moderate fat and protein content (Hinde and Milligan, 2011). Unfortunately, there are few published data on

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nutrient composition of great ape milk, especially whether it varies across lactation.

For great ape infants (e.g., gorilla, orangutan), milk is the primary if not sole food for an extended period of infancy, providing the only supply of the water, organic nutrients, and minerals during the first 6–12 months of life for gorillas and between 12 and 18 months of life for orangutans (van Noordwijk, Kuzawa, & Schaik, 2013; van Noordwijk, Willems, Atmoko, Kuzawa, & van Schaik, 2013). Preferentially, newborn apes in captivity will suckle from their mothers, however, in cases when the mother's milk is not available (e.g., maternal death, infant rejection), zoo nutritionists/veterinarians are responsible for formulating a milk replacer to maximize the chances of survival of the neonate. To accomplish this, they generally use milk from commercially available species as well as nutritional supplements and other additives. One goal of this research is to provide a better understanding of the nutritional profile of milk in two species of great apes in order to assist the professionals charged with their care in captivity to develop improved hand-rearing protocols.

Less is known about immunological components of anthropoid milks, except for data from human milk. Secretory immunoglobulin A (sIgA), the major immunoglobulin present in primate milk, appears to be present in high concentrations in human milk at all stages of lactation, substantially higher than appears to be the case for macaque milk (Milligan, 2007; Hinde and Milligan, 2011). It has been suggested that the higher concentrations of sIgA in human milk could represent an adaptive change in human milk to increase its immunological function, possibly in response to higher pathogen loads derived from cultural/technological advances such as agriculture and the domestication of animals (Milligan, 2007). However, to test this hypothesis, comparison with the milk from our closer relatives, the great apes, is required to rule out the difference in milk sIgA concentration between the monkey and ape lineages as opposed to a possible genus *Homo* evolved trait. Another objective of the present study is to identify the sIgA profiles in milk from gorillas and orangutans throughout lactation and examine how the milk of these two species differ from each other and from published data regarding human milk. Finally, milk constituents often co-vary. The relationship between the nutritional and immunological components of milk have rarely been studied together. The dataset analyzed in this study allows an investigation of associations among these milk constituents, and how they do or do not co-vary over lactation.

2 | METHODS

The research was approved in accordance with the regulations and guidelines set forth by the Animal Care and Use Committees from the Smithsonian National Zoological Park, Zoo Atlanta, and Fresno Zoo, and was in compliance with the American Society of Primatologists Principles for the Ethical Treatment of Non-Human Primates.

2.1 | Animals

The gorilla and orangutan milk samples were collected from fully alert, unanesthetized females that have been trained to voluntarily (in exchange for food rewards) allow animal care staff to express milk by gentle, manual massage of the nipples. Four female gorillas and three female orangutans were used in the present study. Milk samples were collected between January 2009 and October 2014. A total of 53 samples were collected among all 7 animals (Table 1). Two out of the 53 samples were collected at 3 and 5 days from a single gorilla and were categorized as colostrum samples and analyzed separately. The other orangutan and gorilla milk samples ranged from 0.5 to 22 and 1 to 48 months of lactation, respectively. For the Smithsonian National Zoological Park, many samples were collected from a complete evacuation of the mammary gland but most of the sampled milk were relatively small (under 5 ml). Therefore, analysis of milk volume (amount of milk evacuated from mammary gland at each sampling point) was not performed. All milk samples were aliquoted into individual 1 and 2 ml cryovials immediately after collection and stored at −80°C until further analysis at the National Zoological Park (NZP) in Washington, D.C.

2.2 | Laboratory Analysis

Milk constituents were measured at the Nutritional Laboratory of the Smithsonian NZP using standard methods (Hood, Voltura, & Oftedal, 2009; Oftedal and Iverson, 1995). Briefly, for dry matter content (DM), milk samples were aliquoted, weighed, and dried in a forced convection drying oven for 3.5–4 hr at 100°C and then reweighed (AOAC, 1990). Total nitrogen was determined using a Dumas nitrogen gas analysis procedure using a carbon, hydrogen, and nitrogen elemental gas analyzer (Model 2400, Perkin Elmer, Norwalk, CT). Crude protein (CP) was estimated by multiplying total nitrogen by 6.38

TABLE 1 Animals used and sampling number

Individual	Species	Institution	Number of samples	Range of infant ages (days)
1	*Gorilla gorilla*	Zoo Atlanta	5	1217–1452
2	*Gorilla gorilla*	Zoo Atlanta	7	25–413
3	*Gorilla gorilla*	Zoo Atlanta	14	29–576
4	*Gorilla gorilla*	National Zoo	12	3–1306
5	*Pongo pygmaeus*	Zoo Atlanta	2	15–48
6	*Pongo abelii*	Zoo Atlanta	10	140–638
7	*Pongo abelii*	Fresno Zoo	3	192–264
Total			53	3–1452

(Jones, 1941). Crude milk fat (total nonpolar lipid) was measured by the Röse-Gottlieb procedure, which involves 3 sequential extractions with diethyl ether and petroleum ether following disruption of the milk fat globules with ammonium hydroxide and ethyl alcohol (Hood et al., 2009). Total sugar was analyzed by the phenol—sulphuric acid colorimetric procedure (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Marier and Boulet, 1959) using lactose monohydrate standards. Milk samples were diluted using distilled water prior to sugar analysis. Replicate sugar samples were read at 490 nm with a microplate reader and accompanying software (MRX TC Revelation, Dynex Technologies, Chantilly, VA). Results were multiplied by 0.95 to correct for water of crystallization in the standard. Assays were performed in duplicate.

Gross energy (GE, kcal/g milk), was calculated using the formula: GE = (9.11 kcal/g * % fat + 5.86 kcal/g * % CP + 3.95 kcal/g * % sugar)/ 100. This equation has the potential to overestimate GE by a small amount because it fails to correct for non-protein nitrogen (Perrin, 1958; Oftedal, 1984). However, it has been verified against GE values measured by bomb calorimetry for milk from several species, including rhesus macaques and bongo (*Tragelaphus eurycerus*) (Hinde, Power, & Oftedal, 2009; Petzinger et al., 2014).

Concentration of sIgA in ape milk was determined using a commercial ELISA kit for determination of sIgA in human biological fluids (ZeptoMetrix Co., Buffalo, NY). Briefly, a seven-point serial dilution standard was included (0, 3.91, 7.81, 15.62, 31.25, 62.5, and 125 ng/ml) on each plate. Samples were diluted 1:650 with assay diluent. The assay procedure was performed following the manufacturer's recommendations and both standard and samples were ran in triplicate. A linear equation was obtained by plotting the standard concentrations and the corrected absorbance in the X- and Y-axis, respectively. Intra- and inter-assay variations were 1.2 and 11.6 %, respectively. Concentrations of sIgA per unit of energy (mg/kcal) was estimated as: sIgA (mg/mL) / GE (kcal/g).

2.3 | Statistical Analysis

The PROC MIXED procedure of SAS was used to compute the statistical analysis. Milk samples (excluding colostrum) were binned into 5 stages of lactation according to months postpartum (0 to 6 months, more than 6 months to 12 months, more than 12 months to 18 months, more than 18 months to 36 months, and more than 36 months to 48 months). This binning categories were selected based on biological time points related to infant dependence on milk and their mother for solid food. To compare the effect of species (gorilla or orangutan), data from MPP ≤ 36 were included in the analysis because of missing values for orangutan in the last group (36 < MPP = 48) and were ran as repeated measures. The class variables included animal, species, and MPP. The design followed a complete randomized design model including as fixed effects: species, MPP, and the interaction of species × MPP and the random effect of animal. To include all periods of MPP, the data was run as a non-repeated measure by species where the models included the fixed effect of MPP and random effect of animal. Repeated measures were tested to determine the structure of best fit as indicated by the smallest Schwartz Bayesian information criteria. For all SAS models, the degrees of freedom were estimated with the Kenward-Roger specification in the model statement. Pearson correlation coefficients were estimated using the CORR procedure of SAS to describe the relationships between milk component variables across species and within species. The data for milk sIgA and other milk components in tables, figures, and text are presented as least square mean (LSM) and largest standard error of the mean (SEM). Statistical differences were declared as significant if $P \leq 0.05$ and tendency if $0.05 < P \leq 0.10$. For colostrum sIgA and other colostrum components the data is presented as simple mean ± standard deviation.

3 | RESULTS

3.1 | Species Comparison

This analysis aimed to evaluate the main effect of species and the interaction of species × MPP. All parameters measured in milk, besides sIgA and DM, were affected by the interaction of species × MPP (Table 2). Figure 1 shows the interaction of species × MPP throughout lactation. Neither concentrations of sIgA per unit of milk (mg/L, Figure 1A) nor per unit of energy (mg/kcal, data not shown) differed between species throughout lactation. Crude protein % across MPP was greater ($P < 0.01$) in gorillas compared with orangutans (1.27 vs. 0.85 %) and differences between the species were more marked as MPP increased (Figure 1B). Sugar % was lower in gorilla when compared with orangutan milk at 6 MPP whereas no differences were observed between species at any other time point (species × MPP, $P = 0.02$, Figure 1C). Fat % tended to be lower for gorilla compared with orangutan milk at 12 and 18 MPP (species × MPP, $P < 0.01$, Figure 1D). At 36 MPP, this effect was reversed where gorilla milk had significantly higher fat % compared with orangutan milk. The effect of species on concentration of GE in milk (Figure 1E) is similar to that observed for fat content where gorilla milk tended to have lower GE concentration at 6, 12, and 18 MPP whereas by 36 MPP gorilla milk had more GE than orangutan milk (species × MPP, $P < 0.01$).

We considered the difference between species regarding CP in milk (Figure 1B) to be important enough for further analysis, relaxing some of the statistical constraints from the repeated measures analysis. Accordingly, we examined the difference between gorilla and orangutan milk CP as measured by content (%) or by per energy basis (mg/kcal; Figure 2). Milk samples collected between infant age 25–683 days for gorilla and 15–638 days for orangutan were subjected to analysis of covariance (ANCOVA) with species as the categorical variable and infant age as the covariate. This analysis ignores potential differences between individuals of the same species. Gorilla milk CP adjusted for infant age was greater ($P < 0.01$) than infant-age-adjusted orangutan in both CP expressed as % (Figure 2A) and as mg/kcal (Figure 2B). Infant age was a significant ($P < 0.01$) covariate for milk CP % but not ($P = 0.25$) for CP expressed as mg/kcal. Within species, milk CP, expressed as % and as mg/kcal, from gorillas differed ($P < 0.01$, data not shown) among individuals. However, for orangutans no

TABLE 2 Immunoglobulin A (sIgA) and nutrient composition in milk from gorillas and orangutans throughout lactation[a]

	Species			*P*-value		
Milk components[b]	Gorilla	Orangutan	SEM	Species	MPP[c]	Species × MPP
sIgA						
mg/L	34.05	24.99	4.20	0.17	<0.01	0.23
mg/Kcal of GE	0.07	0.05	0.01	0.14	<0.01	0.31
DM, %	10.84	12.32	0.57	0.26	0.07	0.19
CP, %	1.27	0.85	0.06	<0.01	<0.01	0.01
Fat, %	1.78	2.34	0.45	0.40	0.79	<0.01
Sugar, %	6.96	7.30	0.11	0.07	0.07	0.02
GE, Kcal/g	0.51	0.56	0.04	0.41	0.66	<0.01

[a] Animals were kept in captivity at different US zoos and were voluntarily milked in exchange of food.

[b] sIgA and nutrient composition (DM, dry matter; CP, crude protein; GE, gross energy) were measured in non dried milk.

[c] MPP, month postpartum. For this analysis, samples throughout lactation were grouped in 4 groups 0 < MPP ≤ 6, 6 < MPP ≤ 12, 12 < MPP ≤ 18; 18 < MPP ≤ 36.

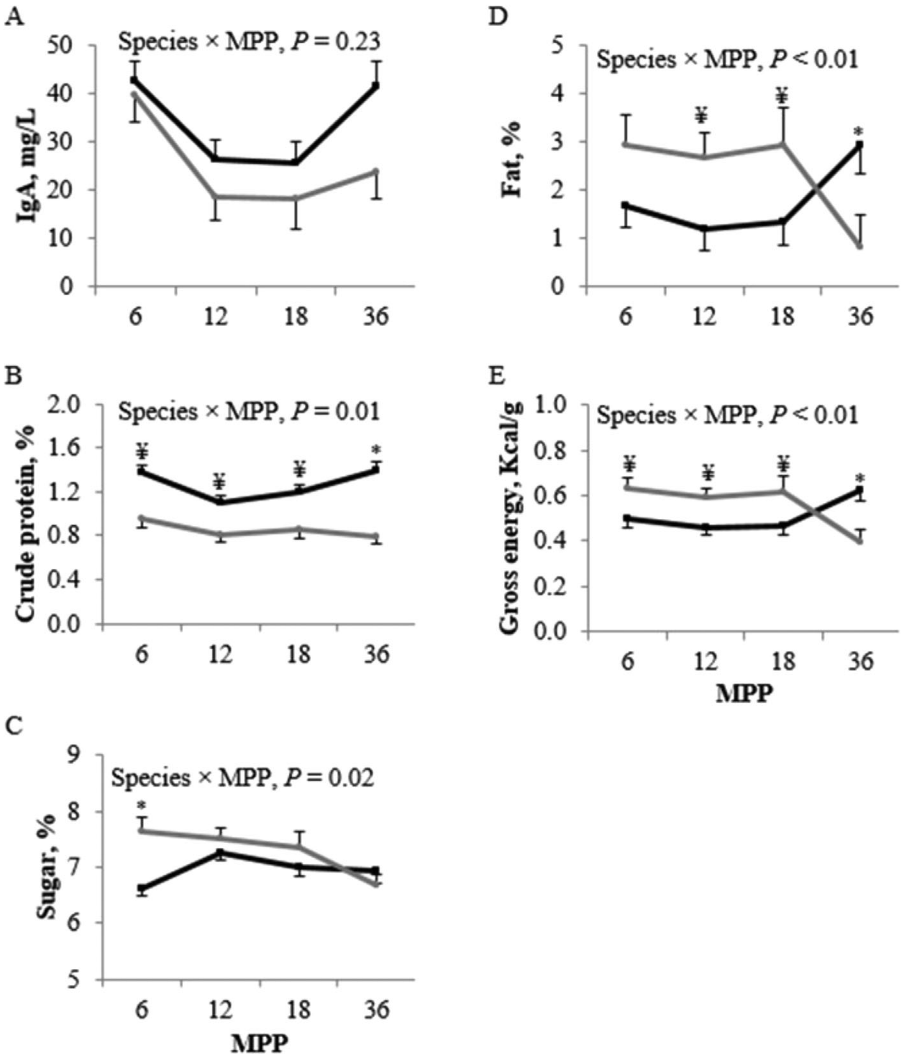

FIGURE 1 Secretory immunoglubin A (sIgA) concentration (A), crude protein % (B), sugar % (C), fat % (D), and gross energy concentration (E) in milk from gorillas (dark line) and orangutans (gray line) throughout lactation. Animals were kept in captivity at different US zoos and were voluntarily milked in exchange of food. Symbols* and ¥ indicate differences ($P \leq 0.05$) and trend towards difference ($P \leq 0.10$) between species at any given time point, respectively

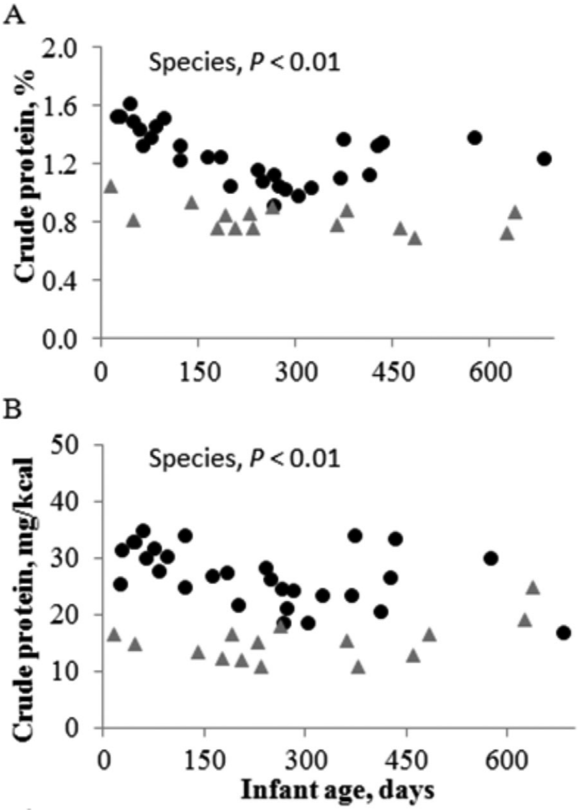

FIGURE 2 Crude protein % (A) and as per energy basis (B, mg/kcal) in milk from gorillas (dark circles) and orangutans (gray triangles) collected between 15 and 683 days of infant age. Animals were kept in captivity at different US zoos and were voluntarily milked in exchange of food

differences were observed among individuals in milk CP measured in either % or mg/kcal.

3.2 | Effect of Months Postpartum Within Species

Figure 3 shows the effect of MPP on sIgA and other nutrients in gorilla milk. Concentration of sIgA was 42.7 mg/L at 6 MPP and declined by 12 and 18 MPP (Figure 3A); however, by 36 MPP sIgA concentrations increased reaching its highest concentration at 48 MPP (79.2 mg/L). Similarly, concentrations of sIgA per unit of energy were higher at 6 MPP (0.09 ± 0.01 mg/Kcal) lower at 12, 18, and 36 months (average 0.06 ± 0.01 mg/kcal) and the highest at 48 MPP (0.20 ± 0.01 mg/kcal; data not shown). Crude protein % (Figure 3B) increased as lactation progressed with peak content observed at 48 MPP. Sugar % in gorilla milk was lower by 48 MPP ($P < 0.01$, Figure 3C). Dry matter % was quite variable at a given MPP, with the peak reached by 36 MPP ($P < 0.01$, Figure 3D). Fat % and GE concentration in gorilla milk was similar to that observed for DM, where peak of fat content ($P < 0.01$, Figure 3E) and GE concentration ($P < 0.01$, Figure 3F) were observed by 36 MPP.

Figure 4 shows the effect of MPP on sIgA and other nutrients in orangutan milk. Orangutan milk sIgA concentration per unit of milk was highest at 6 MPP ($P < 0.01$; Figure 4A), whereas concentrations per unit of energy where similarly higher at 6 and 36 MPP (0.06 ± 0.01 mg/kcal) and lower at 12 and 18 MPP (0.03 ± 0.01 mg/Kcal). Crude protein % was not altered by MPP and averaged 0.85 ± 0.08% throughout MPP (Figure 4B). Sugar % decreased with MPP, with the lowest content observed at 36 MPP (6.67 %, Figure 3C). Neither DM nor fat % were affected by MPP (Figure 4D and Figure 4E, respectively) and averaged 12.22 ± 1.40 and 2.33 ± 1.08% across MPP, respectively. Concentration of GE in orangutan milk tended to decrease by 36 MPP ($P = 0.07$, Figure 4F).

3.3 | Composition of Colostrum from Gorillas

Table 3 shows the average composition of 2 colostrum samples, collected at days 3 and 5 after parturition from a single gorilla. Average sIgA concentration was 68.4 ± 0.8 and 0.13 ± 0.03 mg/L and mg/kcal, respectively. Total DM averaged 12.3 ± 1.5 %, sugar % averaged 5.7 ± 0.5 %, whereas CP and fat % were similar (2.4 ± 0.2 and 2.1 ± 1.3 %, respectively), with a mean GE of 0.56 ± 0.11 Kcal/g.

3.4 | Correlations Between Milk Components

Pearson correlations between milk components is shown in Table 4. Across species, GE was positively correlated with DM ($r = 0.78$, $P < 0.001$), fat ($r = 0.98$, $P < 0.001$), sugar ($r = 0.37$, $P < 0.01$), and negatively correlated with CP ($r = -0.27$, $P = 0.06$). Further, sugar was negatively correlated with sIgA ($r = -0.80$, $P < 0.001$), CP ($r = -0.77$, $P < 0.001$), and positively correlated with DM ($r = 0.24$, $P = 0.08$) and fat ($r = 0.25$, $P = 0.07$). Also, fat was positively correlated with DM ($r = 0.81$, $P < 0.001$) and negatively correlated with CP ($r = -0.29$, $P = 0.04$). Finally, CP was positively correlated with sIgA ($r = 0.82$, $P < 0.001$) and negatively correlated with DM ($r = -0.32$, $P < 0.05$). Similar relationships were observed within the gorilla milk samples. The minimal/lack of relationships observed within orangutan samples may be partly attributed to the low sample numbers.

4 | DISCUSSION

The composition of milk from anthropoid primates can be characterized by having low fat and CP contents and high sugar content, where fat is negatively correlated with sugar and positively correlated with GE (Hinde and Milligan, 2011). A very limited number of studies have evaluated the nutritional composition of milk from great apes such as gorillas and orangutans, which are summarized elsewhere (Hinde and Milligan, 2011). These studies only evaluated samples for a given lactation period (primarily mid-lactation, i.e., milk obtained between 100 and 400 days of infant age) often with only a single sample from a few individuals. There are no published studies of which we are aware that have examined sIgA concentrations in milk from any great ape species. Our study included milk samples throughout the multiyear lactation period characteristic of great apes (van Noordwijk, Willems et al., 2013) with multiple samples from several individuals of each of the two species.

Milk volume was not measured in this study because of the logistical difficulty associated with the collection of milk from unanesthetized captive primates. Research measuring milk volume in non-human great apes is lacking. A few studies have measured milk

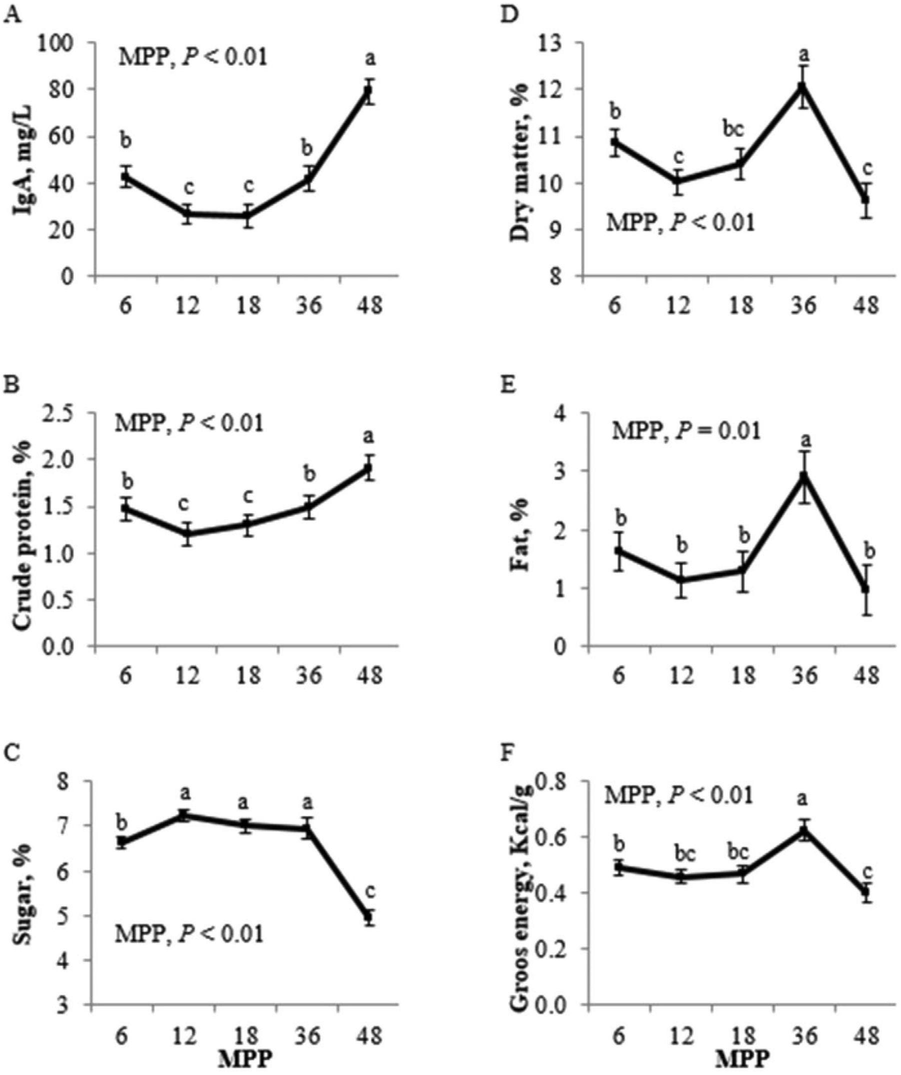

FIGURE 3 Effect of months in milk (MPP) on secretory immunoglubin A (sIgA) concentration (A), crude protein % (B), sugar % (C), dry matter % (D), fat % (E), and gross energy concentration (F) in milk from gorillas. Animals were kept in captivity at different US zoos and were voluntarily milked in exchange of food. Different letters differ ($P \leq 0.05$)

volume during sampling days (Ota, Makino, & Kimura, 1991) or relative milk yield (amount of milk collected after ~ 4 hr of mother and infant separation to avoid nursing [Hinde et al., 2009]) in monkeys. Most of the research regarding milk volume and factors affecting it have been investigated in humans. Hinde and Milligan (2011) concluded that major factors leading to variations in milk synthesis are derived mostly from cultural variations (e.g., nursing behavior, supplementary food during breastfeeding, and use of formulas) and extreme variations in evaluated populations (extremely marginal versus western societies). Most of the variations within population type come from mother's body weight (Brown, Akhtar, Robertson, & Ahmed, 1986), body fat (Barbosa, Butte, Villalpando, Wong, & Smith, 1997), and age (Motil, Kertz, & Thotathuchery, 1997). Therefore, it is logical to imply that such factors may also influence milk volume in non-human great apes. To better understand how variations in milk composition (nutrients and sIgA) affect infant growth and development, further research should prioritize measures of total milk production and factors affecting milk synthesis and its utilization by nursing infants.

4.1 | Milk Nutrients

Our results are consistent with previously published data on gorilla and orangutan milk, describing a milk that is high in sugar (and hence water) and low in fat and CP. For example, the mean milk macronutrients of a single mid-lactation milk sample each from seven healthy wild mountain gorillas (*Gorilla beringei beringei*) were 1.9 ± 0.7% fat, 1.4 ± 0.3% protein, and 6.8 ± 0.9% total sugar, respectively, with protein accounting for 16% of milk energy (Whittier, Milligan, Nutter, Cranfield, & Power, 2010). These values are very similar to the values for *Gorilla gorilla* found in this study (Table 2); on average, protein provided 15% of milk GE in these lowland gorilla milk samples.

Orangutan milk macronutrient composition appears to differ from that of gorilla milk primarily in the protein fraction, with only about 9% of milk GE derived from protein, on average. Crude protein across MPP was lower in orangutan milk compared with gorilla milk, though our rigorous statistical analysis only found this to be significant for milk by 36 MPP and a trend towards significance for MPP < 36 (Figure 1B).

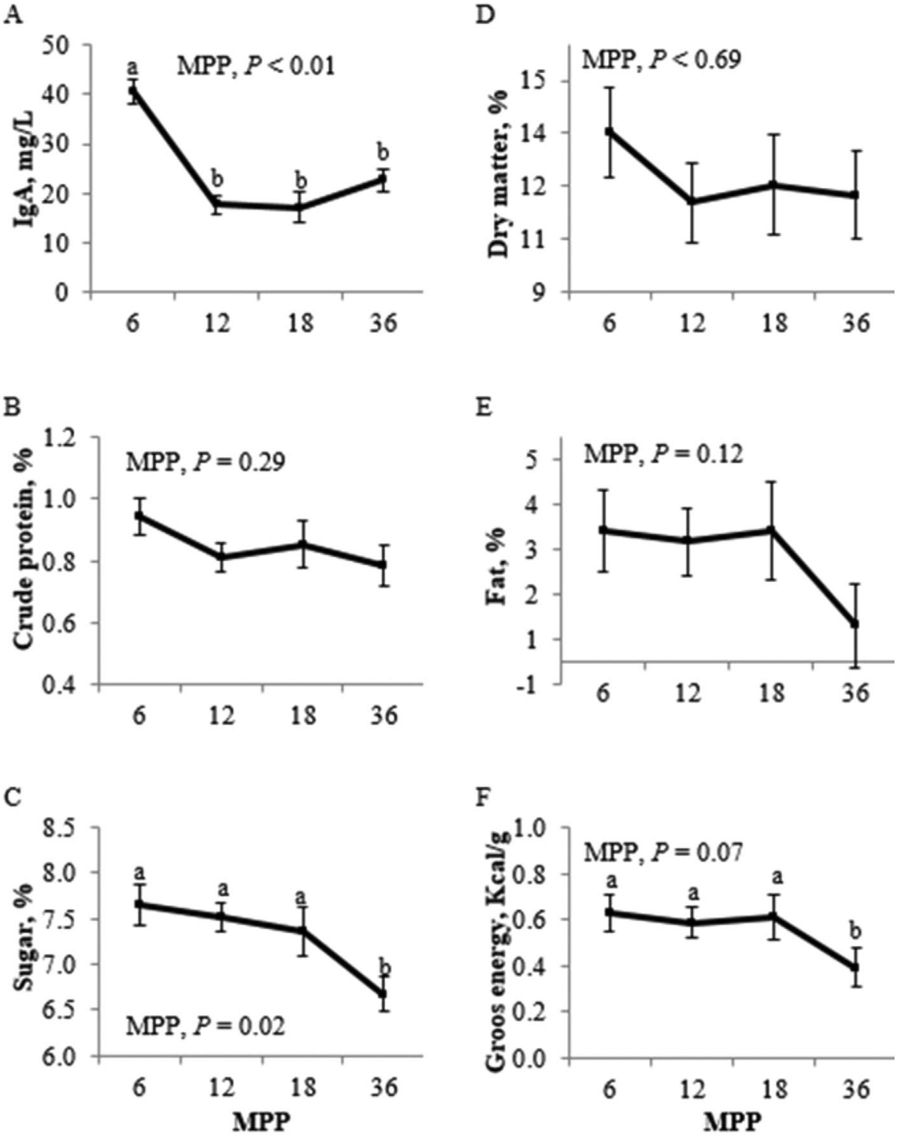

FIGURE 4 Effect of months in milk (MPP) on secretory immunoglubin A (sIgA) concentration (A), crude protein % (B), sugar % (C), dry matter % (D), fat % (E), and gross energy concentration (F) in milk from orangutans. Animals were kept in captivity at different US zoos and were voluntarily milked in exchange of food. Different letters differ ($P \leq 0.05$)

Our ANCOVA results further suggest that orangutan milk is lower in CP. Accordingly, we suggest that milk replacer formulas for orangutans and gorillas should differ in CP content. Based on mean values for samples between 15 and 683 days of infant age a suggested formula for orangutan milk replacer would be: 0.8% CP, 2.6% fat, and 7.3% sugar, with total DM of 12–12.5%. For gorilla milk replacer the suggested values would be: 1.3% CP, 2% fat, and 7% sugar, with a total DM of 11–12%. The orangutan formula should provide about 15 mg/kcal of CP while the gorilla formula should provide 21–26 mg/kcal of CP (reflecting the greater variation observed in the gorilla milk samples; Figure 2).

The higher amount of mg/kcal of CP in gorilla milk is consistent with published data on growth rates in gorilla and orangutan neonates. In the first year of life, growth velocities of gorilla infants are significantly greater than that of orangutan infants (Leigh and Shea 1995, 1996). The amount of CP relative to milk GE appears to relate to relative growth rate in anthropoid primates (Power, Oftedal, & Tardif, 2002; Power, Verona, Ruiz-Miranda, & Oftedal, 2008). New World monkeys have the most CP per GE (approximately 30 mg/kcal) and the fastest growth rates, Old World monkeys have intermediate growth and CP per GE (more than 20 mg/kcal), and the slow-growing humans and great apes (excluding gorillas) have the lowest values of CP per GE (15–18 mg/kcal) for humans, orangutans and chimpanzees [Power et al., 2002, 2008]. Gorillas appear to have faster growth rates than the other great apes and humans [Leigh and Shea 1995, 1996] which seems to be reflected in an increased amount of CP relative to energy in their milk.

In comparison to human milk, milk composition of orangutans and gorillas is roughly similar, but with possible differences in the amounts of CP and fat. Human milk composition data from 8 countries, across lactation lengths of up to 40 months, averaged 1.0% CP, 3.7% fat, and 7.2% sugar (Quinn, Largado, Power, & Kuzawa, 2012). Thus, it would

TABLE 3 Immunoglobulin A (sIgA) and nutrient composition in colostrum for one gorilla after parturition[a]

	Days after parturition		
Milk components[b]	3	5	Average
sIgA			
mg/L	67.9	69.0	68.4
mg/Kcal of GE	0.11	0.14	0.13
DM, %	13.4	11.3	12.3
CP, %	2.58	2.30	2.4
Fat, %	3.02	1.21	2.2
Sugar, %	5.36	6.0	5.7
GE, Kcal/g	0.64	0.48	0.56

[a] Animal was kept in captivity at different US zoos and was voluntarily milked in exchange of food.

[b] sIgA and nutrient composition (DM, dry matter; CP, crude protein; GE, gross energy) were measured in non dried milk.

appear that human, orangutan, and gorilla milks are similar in containing about 7% sugar, but that human milk may contain a higher fat content and an intermediate CP content between orangutans and gorillas. However, the pattern changes when milk CP is expressed as mg/kcal, with both human and orangutan milk containing about 15 mg/kcal of protein, while the data from the gorilla samples in this study imply a significantly higher level of about 25 mg/kcal. Interestingly, the lower protein-per-energy of human milk appears to be a result of an evolved increase in fat and hence energy content of milk, while orangutans have evolved a similar level of protein-per-energy by reducing the CP content.

In some species, milk composition changes as milk is drawn from the mammary gland, with what is termed foremilk typically having lower fat content than the hindmilk. Most of the milk samples assayed in this study were relatively small (under 5 ml) and likely represent foremilk. We cannot be certain that larger samples might not have resulted in a changed composition, especially for fat content. However, the foremilk-hindmilk difference is not universal among mammals (Petzinger et al., 2014). For the National Zoo gorilla in this study, many samples that were collected were from a complete evacuation of the mammary gland. There was no effect of whether the mammary expression was full or partial for any measured milk nutrient or the hormones adiponectin, epidermal growth factor, and transforming growth factor β-2 (Power et al., 2016). The composition of a large milk sample (>30 ml) collected from the *Pongo pygmaeus* female under anesthesia from a prior birth (Milligan, 2007) was identical to the composition of the two small samples collected for this study. The current weight of evidence favors a minimal effect of fore- versus hind-milk on great ape milk composition.

4.2 | Concentrations of Immunoglobulin A

Human and non-human primates are very prone to mucosal infections in early life (e.g., digestive, and respiratory) which increase their odds for mortality and morbidity (Loureiro et al., 1998). Therefore, provision of secretory antibodies, via milk consumption, is critical to minimize the risk of pathogen invasion. Specifically, sIgA plays an important role

TABLE 4 Pearson correlations between milk components across species and within species[a]

Measure[b]	Dry matter	Crude protein	Fat	Sugar	Gross energy
Across species (*n* = 53)					
sIgA	−0.21	0.82***	−0.12	−0.80***	−0.16
Dry matter		−0.32*	0.81***	0.24^{+}	0.78***
Crude protein			−0.29*	−0.77***	−0.27^{+}
Fat				0.25^{+}	0.98***
Sugar					0.37**
Gorilla (*n* = 38)					
sIgA	0.08	0.82***	0.11	−0.87***	0.00
Dry matter		0.12	0.87***	−0.01	0.88***
Crude protein			−0.04	−0.80***	−0.07
Fat				0.03	0.97***
Sugar					0.20
Orangutan (*n* = 15)					
sIgA	0.09	0.50^{+}	0.00	0.25	0.06
Dry matter		0.38	0.61*	−0.03	0.58*
Crude protein			0.21	0.40	0.30
Fat				0.32	0.99***
Sugar					0.47^{+}

$^{+}P < 0.10$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

[a] Animals were kept in captivity at different US zoos and were voluntarily milked in exchange of food.

[b] sIgA and nutrient composition were measured in non dried milk.

inhibiting initial pathogen colonization (Brandtzaeg, 2003). Formula fed babies, who receive no milk sIgA, are at higher risk for illness in western populations. In a study of indigenous Toba women in northeastern Argentina, lower concentration of sIgA in milk was associated with more symptoms of illness (especially gastrointestinal illness) in the breastfed infants (Breakey, Hinde, Valeggia, Sinofsky, & Ellison, 2015). These finding are consistent with sIgA having a protective effect against pathogens (Breakey et al., 2015).

Despite its potential importance, studies evaluating sIgA concentration in non-human apes are lacking, whereas data regarding sIgA concentration is available for humans from a wide range of populations (Breakey et al., 2015; Goldman, Garza, Nichols, & Goldblum, 1982; Hennart, Brasseur, Delogne-Desnoeck, Dramaix, & Robyn, 1991; Kawano and Emori, 2013; Miller and McConnell, 2015; Olivares et al., 2015; Smilowitz et al., 2013; Weaver, Arthur, Bunn, & Thomas, 1998). In a study of Japanese women, milk sIgA was greater than 2,000 mg/L within a few days of parturition, declining to about 1,000 mg/L by 8–12 wk postpartum (Kawano and Emori, 2013). Mean milk sIgA values ranged from 308 to 855 mg/L in the study of Toba women (Breakey et al., 2015). Mean values for milk sIgA in rural Kenyan women were 842 µg/mL, compared with salivary sIgA levels of 324 mg/L, and exhibited a U-shaped distribution with time from birth (Miller & McConnell, 2015). Human milk contains high concentrations of sIgA, which also appear to vary extensively both within and across populations. Some of the variation likely is associated with the time since birth, with sIgA concentration declining over the first 6-to-9 months of lactation, but then appearing to increase if women continue to lactate past one year (see Table 1 in Miller and McConnell [2015]). Other sources of variation likely relate to differences between women, whether genetic or environmental. Regardless, human milk sIgA concentration far exceeds that of gorilla and orangutan milk.

Between gorillas and orangutans, the concentration of sIgA did not statistically differ, although gorilla milk had a numerically 36% greater concentration than that of orangutan milk. However, concentrations of sIgA were highly variable among individual milk samples. The major factor underlying the variation was MPP, as verified for its significant effect when evaluating each species independently. Orangutan and gorilla milk contain a lower concentration of sIgA compared with human milk. The concentration of sIgA across gorillas and orangutans, for the first 36 MPP, was 29.5 mg/L which is about 20–30 times lower than the concentrations of sIgA in human mature milk (Goldman et al., 1982; Kawano and Emori, 2013; Miller and McConnell, 2015).

In lactating women, a number of factors appear to influence the concentration of sIgA in milk. Women who experienced gestational diabetes produce milk with lower-on-average sIgA content (Smilowitz et al., 2013). Mothers with celiac disease also produce milk with lower sIgA concentration (Olivares et al., 2015). The gorillas and orangutans in this study were all healthy, with no signs of disease, either acute or chronic, that may have affected their milk sIgA concentration.

The extensive evaluation of sIgA concentrations in milk and other human fluids have spread the availability of commercial kits for human sIgA, but kits specific for non-human primates are lacking. An early study (Cole and Bowen, 1976), using antihuman sIgA radial immunodiffusion plates, reported a broad range of sIgA, 330–4,620 mg/L (average 241.6 mg/L) in milk from monkeys (*Macaca fascicularis*). More recently (Permar et al., 2008, 2010), using a monkey-specific sIgA ELISA kit to evaluate milk from monkeys (*Rhesus macaque*), reported an average log value of 2.25 (range: 1.5–3 log) which result in an average log-transformed concentration of 178 mg/L. We acknowledge that the use of a human sIgA kit in the current study may not detect the actual total amounts of sIgA in milk from orangutans and gorillas. However, based on data described for monkeys, we are certain that the current results reflect that milk of non-human great apes (gorilla and orangutan) have lower concentrations of sIgA compared with human milk.

We suggest that the high concentration of sIgA in human milk implies a period of increased disease and pathogen exposure to unweaned infants after the divergence from the great apes. Certainly the increased population density and cultural changes from the advent of agriculture and domestication of animals would have increased pathogen exposure, both due to the concentration of human specific pathogens in the environment because of constant human occupation and the associated sanitary issues, as well as the introduction of novel pathogens from the animals being domesticated. The associated increase in infectious diseases appears to have imposed a selective pressure for increased immune factors (e.g., sIgA) in milk as a means to increase neonatal and infant survival (Milligan, 2007). Increases in output of sIgA in milk may be a combined effect of increased maternal synthesis, transfer from maternal circulation to mammary gland, and increased total milk output.

Although, we acknowledge the limitation of having only two samples of colostrum from a single gorilla, they are representative of first breast secretion, as others have reported when characterizing colostrum composition in humans (Jevinen, Laine, Jevenpe, & Suomalainen, 2000) and non-human primates (Urashima et al., 2009). In the current study, the concentration of sIgA in gorilla colostrum was 2 times greater than the average concentration for gorilla milk (≤ 36 MPP), however, after 18 MPP concentrations of sIgA increased to reach its highest concentration at 48 MPP (16% greater than that of colostrum). For orangutans, we were not able to obtain samples beyond 22 MPP, but from the available data, concentrations of sIgA in milk show a numerical trend to increase beyond 18 MPP. A similar pattern was observed in a human study measuring sIgA in milk for 12 months where concentrations of sIgA were 2,000 mg/L at 2–3 days declining to 1,000 mg/L by 4 wk and to 500–600 mg/L by 12–24 wk, to later increased to 900–1,000 mg/L by 36–52 wk (Goldman et al., 1982). In a study of rural Kenyan women, sIgA mean levels were above 900 mg/L in the first three months, declined slightly to just under 800 mg/L at 4–6 months postpartum and then steadily increased through 18 months. Values of milk sIgA were highest in samples collected after 19 months, averaging over 1,000 mg/L (Miller and McConnell, 2015). However, milk was collected from a different group of donors at each evaluated period in these studies, which may bias the results if we consider that others have reported no change in sIgA in milk from lactating mother (> 1 to 18 months), averaging 1,800 mg/L (Hennart et al., 1991) or ranging from 625 mg/L at 9 wk to 746 at 52 wk of lactation (Weaver et al., 1998).

Potential reasons for the observed pattern for sIgA concentrations in non- human apes compared with other species may be: 1) milk output decreased drastically after 36 MPP due to reduced suckling by offspring, concentrating the amount of sIgA per unit of milk; 2) total sIgA secreted per unit of milk increases as a maternal mechanism to ensure greater provision of this critical immune molecule; or 3) a combination of decreased milk output and an increase in sIgA secreted via milk. A study performed in Gambia evaluated the concentrations of sIgA in milk from women during the period 8–52 wk of offspring age, reported that that the amount of milk ingested did not correlate with sIgA concentration (Weaver et al., 1998). The lack of correlation of milk output with sIgA suggests that the sIgA concentration increase after 18 MPP in great apes may be due to a greater amount of sIgA secreted per unit of milk.

The lack of data regarding milk volume in this study, coupled with the limited number of samples in each species at a given MPP limit our ability to draw strong associations between patterns of IgA concentration and total IgA output. However, if the pattern of sIgA concentration observed in this study reflects the biology of non-human great apes, we question why concentrations of sIgA are greater by mid- to late lactation period rather than in early lactation when newborn infants have a more naïve immune system? The great ape offspring starts eating solid food between 6 to 12 months of age (van Noordwijk, Kuzawa et al., 2013; van Noordwijk, Willems et al., 2013). Moreover, by 18 months of age they are more interested in searching for their own food and separating from their mother for longer distances and time (Nowell and Fletcher, 2006; Nowell and Fletcher, 2007). After birth, infants are continuously exposed to new microbes that may reach the gastrointestinal tract via food intake (Mackie, Sghir, & Gaskins, 1999). Food (e.g., leaves, fruits) exposed to the environment, where microbial population abounds, would certainly contain a greater microbial count and novel microbial populations than nursed milk, and may potentially induce gastrointestinal microbial imbalance for which a more responsive immune system is critical to avoid disease occurrence. Hence, a possible explanation is that older great ape infants (after 18 months of age) need a boost in their immune system as they increase their intake of solid food and become more mother-independent, facing new environments with potential greater microbial population.

The results of the present study are indicative that specific milk proteins or other milk components (e.g., oligosaccharides, immune molecules, and hormones) may differ among closely related species. For example, human, gorilla and orangutan milk differ from each other in the concentrations of adiponectin, epidermal growth factor, and TGF-β2 (Power et al., 2016). The results indicate differences in CP content between gorillas and orangutans. This difference may be partly attributed to MPP, but in general, gorilla milk has higher protein content than orangutan milk or any published data regarding human milk samples. Human milk appears to have at least a ten-fold higher concentration of sIgA than do milks from either of these great ape species. A better understanding of these differences will be useful to understand how specific environmental pressures in humans may have selected for a divergent milk composition from that of other primates.

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