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MILK COMPOSITION OF INDIAN RHINOCEROS (*RHINOCEROS UNICORNIS*) AND CHANGES OVER LACTATION

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Abstract: The objective of this study was to determine the major nutrient composition of Indian rhinoceros milk (*Rhinoceros unicornis*) over the first 13 mo of an 18-mo lactation period and to compare the results to those of previous studies on rhinoceros, African elephant (*Loxodonta africana*), and horse milk (*Equus ferus caballus*). The following parameters were measured: dry matter (DM), crude ash (ASH), crude protein (CP), ether extract (EE), nitrogen-free extract (NFE; calculated), lactose, calcium (Ca), phosphorus (P), magnesium (Mg), fatty acids (FAs), and gross energy (GE). DM, ASH, CP, and EE were determined with a proximate analysis, lactose with infrared spectroscopy and an enzymatic method, minerals with an autoanalyzer, FA with gas chromatography, and GE with bomb calorimetry. Milk samples were collected from two Indian rhinoceros cows from Zoo Basel. Rhino A gave birth to her third calf on 10 September 2012; three samples were collected and analyzed (colostrum, milk 1 wk and 2 wk postpartum). Rhino B gave birth to her eighth calf on 05 October 2013; samples were collected and 15 were chosen for the analyses (from colostrum to 13 mo postpartum). The composition of rhino B's colostrum was 13.8% DM (wet-weight basis), 4.8% ASH, 61.8% CP, 0.7% EE, 32.6% NFE, 26.7% lactose, 0.59% Ca, 0.54% P, 0.2% Mg (DM basis), and 20.3 MJ GE/kg DM. Rhino B's sample collected 13 mo postpartum averaged 8.0% DM (wet-weight basis), 3.6% ASH, 16.3% CP, 1.8% EE, 78.3% NFE, 84.7% lactose, 0.54% Ca, 0.48% P, 0.09% Mg (on DM basis), and 17.43 MJ GE/kg DM. The main FAs in rhino B's and rhino A's samples were C10 : 0, C12 : 0, C16 : 0, C18 : 1n9c, and C18 : 2n6c. Milk of the Indian rhinoceros is low in fat and protein but high in lactose, which is comparable to the milk composition of other rhinoceros species and horses, but not African elephants.

Key words: Changes over lactation, colostrum, hindgut fermenters, Indian rhinoceros (*Rhinoceros unicornis*), milk, milk composition.

INTRODUCTION

Since the first birth of an Indian rhinoceros (*Rhinoceros unicornis*) at Zoo Basel in 1956, the zoo has successfully raised 34 calves. This success has provided opportunities for collection and analysis of colostrum and milk from lactating cows. Colostrum and early-lactation milk were collected from a lactating cow in 2012. After the birth of the 34th calf sequential milk samples were collected for the first 13 mo of an 18-mo lactation period beginning in October 2013.

Articles on the composition of Indian rhinoceros milk have already been published, including only limited numbers of samples.[17,18,26] To the authors' knowledge, a study based on milk samples collected over a 13-mo period of lactation has never been presented.[17,18,26] Analysis of milk composition over time will help guide the production of an optimal milk replacer for this species. The current milk replacer used at Zoo Basel is an equine milk replacer with added sucrose (Schweizer, pers. comm.).

The aim of this study was to analyze components in milk samples of two Indian rhinoceros and to compare the results to other rhinoceros species and hindgut fermenters with a similar biology such as horses (*Equus ferus caballus*) and African elephants (*Loxodonta africana*). Additionally, the changes in milk composition over a 13-mo period of lactation were to be determined, in order to complete the preexisting database. The hypothesis was that the milk composition of the Indian rhinoceros is similar to that of the horse.

MATERIAL AND METHODS

Two female Indian rhinoceros were included in this study. Their daily ration consisted of 36 kg dry matter (DM) per day (roughly 2% of body weight). In winter, they received barley straw (*Hordeum vulgare*), hay (mixed and balanced composition, late cut), silaged leaves (oak [*Quer-*

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Table 1. Composition (dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), crude ash (ASH), total sugar, calcium (Ca), phosphorus (P), magnesium (Mg), and sodium (Na)) of the different feedstuffs present in the ration of two female Indian rhinoceros (*Rhinoceros unicornis*) at Zoo Basel.

	Nutrient constituents (% on dry matter basis)									
Feedstuff	DM (% of wet weight)	CP	CF	EE	ASH	Total sugars	Ca	P	Mg	Na
Pellets[a]	98	13.5	18	2.8	10	-[b]	0.85	0.65	-[b]	0.95
Carrots[38]	11.2	6.25	21.4	4.5	9.8	50	0.3	0.2	0.08	0.4
Mineral & vitamin feed[c]	96	3.7	4.4	1.3	70.6	-[b]	15.6	6.25	4.16	5.2
Browse[9,24,d]	-[b]	4–20	12–30	-[b]	3–12	5–15	0.6–4.3	0.06–0.2	0.1–0.65	<0.1
Straw[15,e]	88	3.8	44.2	-[b]	-[b]	-[b]	0.4	0.08	0.09	0.3
Hay[15,f]	86	11	30.2	-[b]	-[b]	-[b]	0.7	0.2	0.1	0.06
Grass[15,g]	22	19.1	26.4	-[b]	-[b]	-[b]	0.5	0.36	0.2	0.05

[a] 3695 Nashorn and Tapir, Protector SA, Kaiseraugst, Switzerland; concentrates based on alfalfa, enriched with minerals and vitamins.
[b] No values.
[c] Mineravit, Dr. E. Graeub AG, Bern, Switzerland (ingredients: minerals, cereal, carob flower meal, fennel, trace elements, vitamins, flavoring substances).
[d] Oak, European beech, sycamore maple, common hazel, willow, European ash.
[e] Barley.
[f] Mixed and balanced composition, late cut.
[g] Mixed and balanced composition, different cuts.

cus robur], European beech [*Fagus sylvatica*], sycamore maple [*Acer pseudoplatanus*], common hazel [*Corylus avellana*], willow [*Salix alba*], and European ash [*Fraxinus excelsior*]), and branches without leaves (cherries [*Prunus avium*], common plum [*Prunus domestica*]); in summer, fresh grass (mixed and balanced composition, different cuts) and fresh leaves and branches were also fed. The diet was the same throughout the year with the exception of roughages (summer, winter). Roughage was 65% of the main diet. The remaining 35% were carrots, concentrates based on alfalfa, and a mineral and vitamin feed (Table 1). Milk samples from the first rhinoceros cow (rhino A, third calf, 19 yr old at calving) were collected 4 hr after parturition, 1 and 2 wk postpartum. A total of three samples were available for analyses. The milk samples of the second rhinoceros (rhino B, eighth calf, 31 yr old at calving) were collected at parturition and over the course of a year. Colostrum (sample taken on calving day) and one sample per week during the first month of lactation were analyzed. Thereafter, one sample per month up to 13 mo postpartum was analyzed. A total of 15 samples were selected for analyses. Rhino B's samples were collected during the night. While the calf drank, the zookeeper approached and milked the remaining teat simultaneously. Milk was collected out of both teats depending on the calf's preference. Thus, the first fraction was always consumed by the calf, resulting in the collection of the middle fraction by the zookeeper. No drugs were used to increase milk flow. Sample sizes ranged from 100 ml (colostrum) to 400 ml (peak lactation). The samples were frozen (–20°C) right after their collection.

The following components were analyzed: DM, crude ash (ASH), crude protein (CP), ether extract (EE), nitrogen-free extracts (NFE, calculated), calcium (Ca), phosphorus (P), magnesium (Mg), and gross energy (GE). Furthermore, frozen whole milk samples were sent out to determine the fatty acid (FA) profile and lactose concentrations. Defrosted milk (125 ml) was lyophilized (Christ Gefriertrocknungsanlage GAMMA 2-16 LSC, 37520 Osterode, Germany) for 72 hr before analysis to guarantee homogeneity for CP, EE, and GE analyses. Prior to analyses, the samples were frozen for a maximum of 33 mo (rhino A) and 20 mo (rhino B).

The DM content was determined after heating 1 g of whole defrosted milk in an oven at 103°C for 4 hr. ASH was measured after ashing in a muffle furnace (Heraeus M110, Heraeus Instruments GmbH, 63450 Hanau, Germany) at 550°C for 13 hr. EE was analyzed as follows: 1 g lyophilized milk of each sample was first boiled in HCl for 60 min with a SoxCap 2047 machine (Foss, 3400 Hillerød, Denmark) for hydrolysis; samples were then dried at 60°C over night; finally a soxhlet fat extraction was performed with petroleum ether and EE was determined after drying the samples at 100°C for 90 min.[40] CP was estimated by the Kjeldahl method.[5] 1 g sample was incinerated

with a Kjeldahl catalyst and sulfuric acid at 420°C for 1 hr 30 min. After that, CP was calculated (N × 6.25) with a fractionating machine (Kjeltec™ 2300 Analyzer Unit, Foss). Minerals were measured in ASH. ASH was dissolved in an 8% HCl solution and centrifuged. Ca, P, and Mg were then determined with an autoananalyzer (Cobas Mira Roche-autoanalyzer, F. Hoffmann-La Roche Ltd., 4070 Basel, Switzerland) in the resulting solution. GE was measured with a bomb calorimeter (IKA® Calorimeter System C2000, IKA®-Werke GmbH & Co. KG, 79219 Staufen, Germany). Samples of approximately 0.4–0.5 g lyophilized milk were used. Each analysis was performed as duplicate with the exception of bomb calorimetry, which was performed in triplicate.

Lactose concentration was determined with Mid-IR-Spectroscopy using a MilkoScan FT 6000 set (Foss) with the predictive model for lactose. The calibration of the MilkoScan was done with dairy cow (*Bos taurus*) milk's long-term standards F1–F4 and E1–E4 of the company QSE GmbH (Qualität-Sicherheit-Entwicklung, 85283 Wolnzach, Germany). The MilkoScan was not validated for the use of nonbovine milk. In some of rhino B's samples (8 and 32 days postpartum as well as 11 mo postpartum) and in all three of rhino A's samples, lactose was measured using an enzymatic method: 1 g liquid milk was weighed in a 100-ml flask; 60 ml distilled water and 5 ml of Carrez-II-Solution ($ZnSO_4 \times 7\ H_2O$, concentration: 30 g/100 ml/100 ml) were added and the flask was spun. After 5 ml of Carrez-I-Solution ($K_4[Fe(CN)_6] \times 3\ H_2O$ concentration: 15 g/100 ml/100 ml) were added, the flask was spun once again and filled up to the mark with distilled water (100 ml). Finally, the solution was filtered with a folded filter (150-mm diameter), and lactose concentration was measured in the solution using photometry (Specord 30 UV-VIS Photometer, Analytik Jena, 07745 Jena, Germany).[4] There was a tight correlation ($r = 0.995$) between the results of the two lactose measurement methods.

FA analysis (caprylic acid [C8 : 0], capric acid [C10 : 0], undecanoic acid [C11 : 0], lauric acid [C12 : 0], myristic acid [C14 : 0], palmitic acid [C16 : 0], stearic acid [C18 : 0], cis-9-oleic acid [C18 : 1n9c], linoleic acid [C18 : 2n6c], alpha-linolenic acid [C18 : 3n3]) was performed with direct transesterification.[20] An aliquot of each sample (100–200 mg liquid) was weighed in a glass tube with a screw top; 2 ml methanol-hexane-mixture, a magnetic stirrer, and 200 µl acetyl chloride were added; the mixture was then heated to boiling point and kept boiling for 1 hr under constant stirring; after that, the solution was cooled in a water bath for 5–10 min and 4 ml of potassium carbonate solution were added. Finally, the content of the glass tube was mixed and centrifuged at 503 × g for 10 min at room temperature. The upper hexane phase was aspirated and FA profile was determined with a gas chromatograph (Trace 1300 Thermo Fischer Scientific, 63303 Dreieich, Germany; SP-2560 Columm 100 m × 0.25 mm × 0.2 m, Supelco, Bellefonte, PA 16823, USA; carrier gas: nitrogen).

NFE DM basis was calculated with the following formula: NFE DM basis = 100 – ASH – CP – EE (all DM basis).[15]

All results of the analyses, except for DM (% on a wet-weight basis) and individual FA (% of total FA) were transformed as percentages on a DM basis.

Rhino B's samples were classified into four lactation periods: colostrum (sample taken on calving day), early-lactation (20–41 days postpartum), mid-lactation (5–7 mo postpartum), and late-lactation (11–13 mo postpartum). Changes between the lactation periods in DM, ASH, CP, EE, NFE, lactose, Ca, P, Mg, and GE were statistically evaluated with an analysis of variance (SigmaStat 4.0, Systat Software Inc., San José, CA 95110, USA). The Holm-Sidak method (for pairwise multiple comparisons of the mean of the different lactation periods) was performed to test for differences between lactation periods (significant at $P < 0.05$).

Rhino A's results are expressed as percentages of Rhino B's results. The same procedure was used to compare the major milk components (DM, ASH, CP, EE, NFE, lactose, Ca, P, Mg, GE, and FA profile) of Rhino B with those of other rhinoceros species, African elephant and horse.

RESULTS

Results of the analyses of DM, ASH, CP, EE, NFE, lactose, Ca, P, Mg, and GE in rhino B's and rhino A's milk samples are shown in Table 2. The comparison of the nutrients found in colostrum and approximately 1 wk postpartum milk of rhino B and rhino A showed that ASH was greater in rhino A's colostrum (131% of rhino B's ASH) and lactose was less in rhino A's colostrum (33.2% of rhino B's lactose). The other nutrients in colostrum had similar concentrations (between 75.5 and 122% of each other's values). Milk samples 5–8 days postpartum showed similar values for the different milk parameters (between 85.3 and 108% of each other's values), except for EE,

Table 2. Analyzed milk composition (dry matter [DM], crude ash [ASH], crude protein [CP], ether extract [EE], nitrogen free extracts [NFE], lactose, calcium [Ca], phosphorus [P], magnesium [Mg] and gross energy [GE]) of two Indian rhinoceros (*Rhinoceros unicornis*), rhino A and rhino B, at different time points postpartum during lactation.

	Milk constituents (% of dry matter)									
Milk samples (days postpartum)	DM (% of w.w.[a])	ASH	CP	EE	NFE	Lactose	Ca	P	Mg	GE (MJ/kg)
Rhino B										
0 colostrum	13.8	4.84	61.8	0.74	32.6	26.7	0.59	0.54	0.20	20.3
8	9.50	4.88	27.4	3.26	64.4	60.7	0.83	0.70	0.17	18.3
20	9.62	4.11	20.6	3.96	71.3	- [b]	0.64	0.58	0.13	18.5
32	8.84	3.78	16.0	4.48	75.7	72.6	0.69	0.43	0.12	18.3
41	8.91	3.57	15.4	6.38	74.7	72.8	0.69	0.41	0.11	18.4
79 (2 mo postpartum)	8.59	3.50	16.0	4.01	76.5	75.8	0.69	0.41	0.09	17.8
109 (3 mo postpartum)	8.59	3.39	13.2	1.87	81.5	- [b]	0.68	0.35	0.09	16.8
141 (4 mo postpartum)	8.20	3.51	13.0	1.69	81.8	81.3	0.69	0.38	0.09	17.1
165 (5 mo postpartum)	8.55	3.38	11.8	4.02	80.8	- [b]	0.58	0.35	0.08	17.4
199 (6 mo postpartum)	8.43	3.33	12.6	4.14	80.0	75.5	0.55	0.37	0.09	17.7
229 (7 mo postpartum)	8.44	3.80	16.0	3.04	77.1	74.6	0.62	0.49	0.09	17.4
313 (10 mo postpartum)	8.08	3.77	14.3	2.79	79.1	76.4	0.48	0.52	0.09	17.3
363 (11 mo postpartum)	7.98	3.67	15.8	3.68	76.9	78.7	0.44	0.55	0.09	17.7
385 (12 mo postpartum)	8.33	3.49	14.1	0.93	81.5	78.1	0.52	0.46	0.09	16.9
404 (13 mo postpartum)	8.02	3.62	16.3	1.79	78.3	84.7	0.54	0.48	0.09	17.4
Rhino A										
0 colostrum	10.4	6.36	59.3	0.83	39.9	8.88	0.57	0.27	0.24	19.6
5 (approx. 1 wk postpartum)	9.78	4.16	24.6	6.14	69.3	59.6	0.74	0.59	0.15	18.3
10 (approx. 2 wk postpartum)	8.91	4.65	- [b]	- [b]	- [b]	64.9	0.79	0.60	0.16	18.4

[a] w.w. = Wet weight.
[b] Not enough material.

which was greater in rhino A's sample (188% of rhino B's concentration).

Table 3 shows the development and changes of rhino B's milk parameters over time. DM concentrations were greater ($P < 0.05$) in colostrum and early-lactation milk compared to mid- and late-lactation milk, where DM concentrations did not differ significantly anymore (Table 3). Colostrum had greater ($P < 0.05$) ASH, CP, and NFE concentrations than early-, mid-, and late-lactation milk (Table 3). EE concentrations increased during the first 41 days postpartum in rhino B's

Table 3. Comparison between the milk composition of a captive Indian rhinoceros (*Rhinoceros unicornis*) (dry matter, crude ash, crude protein, ether extract, nitrogen-free extracts, lactose, calcium, phosphorus, magnesium, and gross energy) in colostrum, milk at early- (20–41 days postpartum), mid- (5–7 mo postpartum) and late-lactation (11–13 mo postpartum).

Milk constituents (% on dry matter basis)	Colostrum ($n = 1$)	Early-lactation milk ($n = 3$)	Mid-lactation milk ($n = 3$)	Late-lactation milk ($n = 3$)
Dry matter (% of w.w.[a])	13.8[bc]	9.12 ± 0.43[c]	8.47 ± 0.07	8.11 ± 0.19
Crude ash	4.84[c]	3.82 ± 0.27	3.50 ± 0.26	3.59 ± 0.09
Crude protein	61.8[c]	17.3 ± 2.84	13.5 ± 2.22	15.4 ± 1.16
Ether extract	0.74	4.94 ± 1.27[c]	3.73 ± 0.60	2.13 ± 1.41
Nitrogen-free extracts	32.6[c]	73.9 ± 2.29	79.3 ± 1.89	78.9 ± 2.36
Lactose	26.7[c]	72.7 ± 0.20	75.1 ± 0.64	80.5 ± 3.61
Calcium	0.59	0.67 ± 0.03[c]	0.58 ± 0.04	0.50 ± 0.05
Phosphorus	0.54	0.47 ± 0.08	0.40 ± 0.06	0.49 ± 0.04
Magnesium	0.11	0.12 ± 0.01	0.09 ± 0.01	0.09 ± 0.00
Energy (MJ/kg DM)	20.3[c]	18.4 ± 0.12[c]	17.5 ± 0.18	17.4 ± 0.43

[a] w.w. = Wet weight.
[b] Note: values are expressed as mean ± SD.
[c] Mean values within a row with a superscript [c] significantly differ ($P < 0.05$) from other lactation time points.

Table 4. Fatty acid (FA) profile (caprylic acid [C8 : 0], capric acid [C10 : 0], undecanoic acid [C11 : 0], lauric acid [C12 : 0], myristic acid [C14 : 0], palmitic acid [C16 : 0], stearic acid [C18 : 0], cis-9-oleic acid [C18 : 1n9c], linoleic acid [C18 : 2n6c], alpha-linolenic acid [C18 : 3n3]) of the milk of two Indian rhinoceros (*Rhinoceros unicornis*) (rhino A, rhino B), at different time points during lactation.

	Fatty acids (% of total FAs)										
Milk samples (days postpartum)	C8 : 0	C10 : 0	C11 : 0	C12 : 0	C14 : 0	C16 : 0	C18 : 0	C18 : 1n9c	C18 : 2n6c	C18 : 3n3	Total FA (mg/l w.w.[a])
Rhino B											
0 colostrum	4.46	28.9	2.85	13.9	5.17	12.9	4.61	18.2	7.97	1.06	762
8	5.26	32.7	1.84	17.7	7.26	9.44	2.71	11.7	9.75	1.74	1611
20	6.05	35.5	1.45	17.6	7.39	7.91	2.99	8.55	9.19	3.39	1556
32	8.33	37.0	1.16	16.3	5.66	7.78	2.60	10.1	9.71	1.42	2173
41	7.73	35.9	1.11	15.0	5.21	8.41	2.77	11.9	10.2	1.83	2819
79 (2 mo postpartum)	7.54	29.3	1.36	12.7	4.29	10.1	3.22	16.3	13.5	1.76	1751
109 (3 mo postpartum)	7.36	24.4	1.72	11.6	3.52	12.1	4.22	21.1	12.7	1.35	912
141 (4 mo postpartum)	7.97	31.1	1.27	13.6	4.36	8.64	3.52	13.4	14.4	1.79	1343
165 (5 mo postpartum)	7.80	30.1	1.20	12.4	4.21	7.74	2.82	12.6	18.2	2.90	1654
199 (6 mo postpartum)	8.43	32.8	1.52	13.5	4.23	6.92	2.38	10.8	16.5	2.89	1850
229 (7 mo postpartum)	8.58	32.3	1.85	12.7	3.95	7.66	3.03	9.07	15.8	5.15	1632
313 (10 mo postpartum)	3.63	9.5	1.54	6.33	2.94	15.3	5.05	26.4	22.6	6.61	738
363 (11 mo postpartum)	6.19	20.8	2.39	10.0	3.76	9.23	4.03	16.9	21.9	4.82	1026
385 (12 mo postpartum)	6.68	17.9	3.02	9.79	2.99	12.8	5.68	24.3	14.9	2.00	338
404 (13 mo postpartum)	7.75	25.0	2.61	11.2	3.83	8.44	3.14	10.7	17.9	9.41	903
Rhino A											
0 colostrum	3.82	25.5	2.14	12.5	4.63	13.8	5.82	21.7	9.76	0.33	943
5 (approx. 1 wk postpartum)	5.37	35.5	1.42	16.6	5.92	8.11	3.09	12.3	10.8	0.96	2887
10 (approx. 2 wk postpartum)	4.83	35.0	1.37	17.6	6.87	7.62	2.63	10.9	12.2	0.96	2505

[a] w.w. = Wet weight.

milk and were greater ($P < 0.05$) in early-lactation milk compared to colostrum (Table 3). The lactose concentration of colostrum was markedly lower than the NFE concentration of colostrum and lower ($P < 0.05$) than early-, mid-, and late-lactation milk. After a significant increase of lactose concentration between colostrum and early-lactation milk, lactose concentrations stabilized at a comparably high concentration (Table 3). Ca concentrations increased ($P < 0.05$) from colostrum to early-lactation milk and then decreased ($P < 0.05$) throughout the rest of the lactation period (Table 3). P and Mg concentrations were similar across the lactation (Table 3). GE concentrations in rhino B's samples were greater ($P < 0.05$) in colostrum and early-lactation milk compared to mid- and late-lactation milk (Table 3).

The FA profile of each milk sample is shown in Table 4. The comparison of the FA profile found in colostrum of rhino B and rhino A showed that except for C18 : 3n3, which was markedly lower in rhino A's colostrum (31.1% of rhino B's concentration), the FA concentrations found in both colostrum samples were similar (between 75.2 and 126% of each other's values). Milk samples 5–8 days postpartum also showed similar values for the different milk parameters (between 77.1 and 114% of each other's concentration), except for C18 : 3n3, which was lower in rhino A's sample (55% of rhino B's concentration).

In Table 4 and Figures 1 and 2, the FA concentrations over time measured in rhino B's samples are described. The results reveal, similar to EE, individual FA concentrations fluctuated over time. The FA with the greatest concentration was C10 :0 with a mean of 28.6% of total FA, followed by C12 : 0 and C18 : 1n9c. Figure 1 shows that C10 : 0 and C12 : 0 decreased in the milk during the summer (between 229 and 363 days postpartum) and that, in contrast, C18 : 1n9c and C18 : 2n6c increased. Figure 2 shows an increase of unsaturated FA and a decrease of saturated FA in milk samples taken during the summer.

The composition of mid-lactation milk of other rhinoceros species, African elephant, and horse is compared to that of rhino B in Table 5. The average DM concentrations (% of wet weight) were very similar in the compared rhinoceros species and the horse (98.2–124% of rhino B's concentration). The African elephant on the other

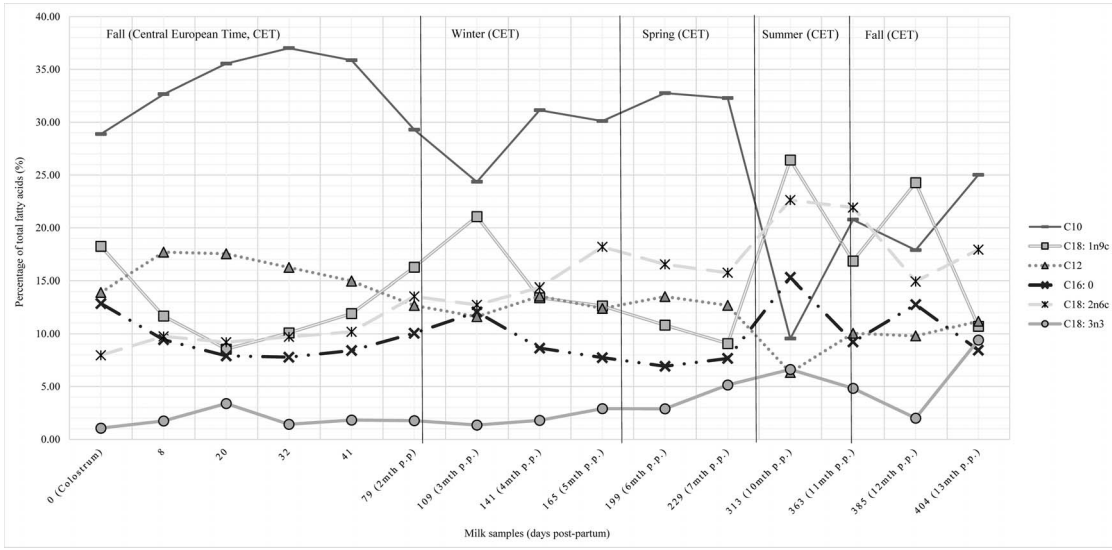

Figure 1. Fatty acid (FA) profile (capric acid [C10 : 0], lauric acid [C12 : 0], palmitic acid [C16 : 0], cis-9-oleic acid [C18 : 1n9c], linoleic acid [C18 : 2n6c], alpha-linolenic acid [C18 : 3n3]) in a captive Indian rhinoceros (*Rhinoceros unicornis*) milk (rhino B, age 31 yr) over 13 mo of an 18-mo lactation.

hand showed approximately double the DM concentrations of rhino B (243%). ASH concentration (% DM) of rhino B was similar to the black rhinoceros (*Diceros bicornis*) and the horse (82.4–79%) but different than the white rhinoceros (*Ceratotherium simum*), which had a lower ASH concentration than rhino B (52.6 %). CP concentrations found in the milk of the Indian rhinoceros, black rhinoceros, white rhinoceros, and horse were similar to rhino B's concentrations (95.2–109%). The Sumatran rhinoceros (*Dicerorhinus sumatrensis*), however, differed with a higher concentration of CP (137%) compared to rhino B. The African elephant milk revealed higher CP concentrations (170% compared to rhino B) as well. EE concentrations were lower in the black rhinoceros milk than in rhino B's (65.8%). The other rhinoceros species, African elephant, and horse showed markedly higher concentrations of EE compared to rhino B's (Indian rhinoceros

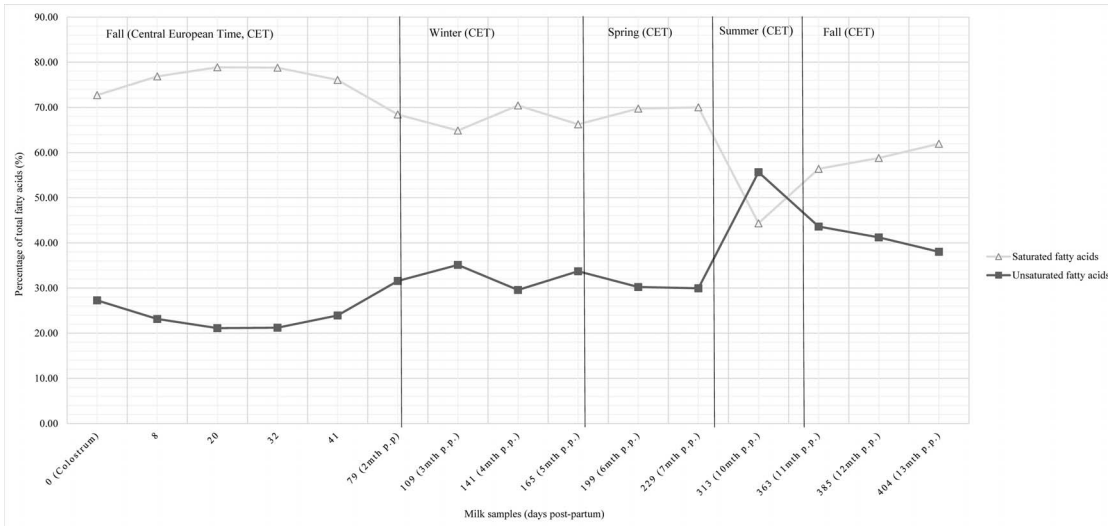

Figure 2. Saturated and unsaturated fatty acids (FAs) in a captive Indian rhinoceros (*Rhinoceros unicornis*) milk (rhino B, age 31 yr) over 13 mo of an 18-mo lactation.

Table 5. Comparison of the major milk composition (dry matter, crude ash, crude protein, ether extract, lactose, calcium, phosphorus, magnesium and gross energy) of a captive Indian rhinoceros (*Rhinoceros unicornis*) with other rhinoceros species (*Diceros bicornis, Ceratotherium simum, Dicerorhinus sumatrensis*) and hindgut fermenters, African elephant (*Loxodonta africana*) and horse, at mid-lactation.

Milk constituents (% dry matter basis)	Indian rhinoceros rhino B	Indian rhinoceros[17,18,26]	Black rhinoceros[2,14]	White rhinoceros[31]	Sumatran rhinoceros[41]	African elephant[30]	Horse[29,34,37]
Dry matter (% of w.w.[c])	8.44 (100)[a]	8.50 (101)	8.80 (104)	8.84 (105)	8.29 (98.2)	20.5 (243)	10.5 (124)
Crude ash	3.80 (100)	-[b]	3.00 (79)	2.00 (52.6)	-[b]	-[b]	3.13 (82.4)
Crude protein	16 (100)	15.2 (95.2)	15.9 (99.5)	17.4 (109)	21.8 (137)	27.2 (170)	17.5 (109)
Ether extract	3.04 (100)	14.3 (469)	2.00 (65.8)	7.00 (230)	8.79 (289)	51.8 (1704)	12.3 (404)
Lactose	74.6 (100)	77.5 (104)	75.00 (101)	73.5 (99)	74.9 (100)	19.7 (26.4)	67.2 (90.2)
Calcium	0.62 (100)	0.87 (138)	0.69 (110)	0.79 (125)	-[b]	-[b]	0.69 (110)
Phosphorus	0.49 (100)	0.25 (51)	0.49 (100)	0.35 (71.4)	-[b]	-[b]	0.53 (108)
Magnesium	0.09 (100)	-[b]	-[b]	0.10 (111)	-[b]	-[b]	0.04 (44.4)
Energy (MJ/kg DM)	17.4 (100)	-[b]	16.6 (95.4)	-[b]	-[b]	-[b]	19.9 (114)

[a] Values in parentheses = % of rhino B's value.
[b] No values found in the literature.
[c] w.w = Wet weight.

469%, white rhinoceros 230%, Sumatran rhinoceros 289%, horse 404%, and African elephant 1704%). Lactose concentrations in milk of the different rhinoceros species and of the horse were similar (90.2–104%); the milk of the African elephant, however, showed distinctly lower concentrations (26.4%). Rhino B's Ca concentration was less than other Indian rhinoceros samples (138% of rhino B's values), but similar to other rhinoceros species, the African elephant, and the horse (110–125% of rhino B's values). P concentrations were similar in milk of other rhinoceros species and horse compared to those of rhino B (71.4–108%). Mg concentrations found in white rhinoceros milk was similar to the milk of rhino B (111%), but concentrations found in horse milk were less (44.4%). Energy concentration of rhino B's milk was similar to values reported for black rhinoceros (95.4%) and horse milk (114%).

DISCUSSION

The development of rhino B's measured milk parameters was similar to those of the other rhinoceros species and horse milk; concentrations of DM, CP, EE, minerals, and energy decreased over time while lactose concentration increased.[29,32,37] The milk parameters of the African elephant developed in the opposite way: concentrations of DM, CP, EE, minerals, and energy increased over the entire lactation period, while lactose concentration decreased.[22,30]

The high CP concentrations in the colostrum of rhino B and rhino A compared to the low concentrations of their milk can be explained by the presence of immunoglobulins, which are extremely high in colostrum and decrease in the days postpartum.[19,23] The rhinoceros has a diffuse epitheliochorial placenta, similar to the horse, which does not allow antibodies to transfer through it.[13] This is why rhinoceros calves and foals have to take up antibodies from colostrum within 24 hr after birth to ensure passive immunity.[13] Asian elephants (*Elephas maximus*) have an endotheliochorial placentation. This type of placenta enables a transplacental antibody transfer from the elephant cow to the calf, so that the majority of the antibody transfer in Asian elephant occurs prenatally.[28] This could also be true for the African elephant and explain why the CP concentration in its milk is higher compared to the colostrum of the Indian rhinoceros in this study.

EE values found in rhino B's milk samples showed fluctuations over the lactation. Several factors may influence EE concentration; these are

well known in cattle and include genetic-heredity factors (slow influence), environment (nutrition, feeding management, time of milking), disease (above all, mastitis), season (changes in type of feed and climatic conditions), feed (intake, frequency, grain and fiber concentrations, physical particle size), stage of lactation, milk fraction, body condition score, and age.[27] A previous study demonstrated that EE is the most variable milk constituent because concentrations vary with dietary changes, whereas CP concentrations are mainly influenced by genetics.[27] Thus, it is important to collect milk samples daily at the same time and feed the animals in question with the same diet.

Because different rhinoceros species do not have the same diets, it could explain the differences among their milk EE concentrations. The black and the Sumatran rhinoceros are browsers, the white rhinoceros is a grazer, and the Indian rhinoceros is a mixed feeder.[36] The cell walls of browse are thinner compared to the cell wall of grasses and in general browse contains a higher proportion of cell content compared to cell wall. Thus it holds higher proportions of highly digestible sugars, proteins, and lipids compared to grasses, which contain a higher concentration of cell walls that are less digestible.[39] Acacia (*Acacia brevispica*) for example, a browse preferred by the black rhinoceros, contains between 1.4 and 1.8% EE (DM basis), whereas the natural diet of the white rhinoceros contains on average 1.1% EE (DM basis).[12,16] However, the black rhinoceros showed lower concentrations of EE in the milk than the white rhinoceros (Table 5). It has to be kept in mind that browse plants differ between continents and thus do not provide the same nutrient composition, which makes a comparison difficult. In this case, the milk of the white rhinoceros was from a free-ranging individual, whereas the black rhinoceros was in human care.[2,14,31] In humans, horses, and some ruminants, the EE concentration increases during the time of milking. The first milk fraction is lower in EE than the last milk fraction.[1] The samples of this study were always taken from the middle fraction, but not exactly at the same time. This could have contributed to the fluctuations found in rhino B's samples. Furthermore, a study in human milk composition showed that EE content is significantly lower in samples collected during the night or morning compared to samples collected during the afternoon or evening.[3]

African elephant milk showed an increase of EE concentration during lactation. One explanation could be that African elephant lactation lasts from 2 to 8 yr and weaning occurs sometimes only when the female gives birth to the next calf. The increase of EE content coupled to the decrease in sugar allows an increase of the nutrient density in milk over the lactation period, which minimizes the maternal water loss. Namely, to fulfill the same nutritional needs of the calf, a smaller milk volume is necessary.[1] Horse foals and rhinoceros calves are weaned earlier, at 12 and 18 mo, respectively.[36] To push the foal and calf to eat roughage earlier could be an explaination of why the EE concentrations of horse and rhinoceros milk tends to decrease over the lactation.

The main FA published for colostrum of the Indian rhinoceros was C26 : 0 (49.3% of total FA) followed by C10 : 0 (35.6% of total FA) which was also the main FA measured in the colostrum in this study (rhino B 28.9 % and rhino A 25.5 % of total FAs).[17] C26 : 0 was not measured in this study. The proportions of C12 : 0, C16 : 0, C18 : 1n9c, and C18 : 2n6c were lower than those measured in rhino B's colostrum (published values: C12 : 0, 0.4%; C16 : 0, 0.36%; C18 : 1n9c, 0.19%; C18 : 2n6c, 0.04%).[17] FA composition of the white rhinoceros in late lactation showed high concentrations of C10 : 0 (25.5% of total FAs), C12 : 0 (16.5%), C16 : 0 (15.8%), and C18 : 1n9c (8.56%), but a lower concentration of C18 : 2n6c (3.71%) and higher concentrations of C14 : 0 (9.57%) and C18 : 0 (8.86%).[31] Colostrum of rhino B showed similarities with that of the Asian elephant, which also contained high proportions of C10 : 0 (29.4% of total FAs), C12 : 0 (18.3%), C16 : 0 (12.6%), and C18 : 1n9c (17.3%).[35] Hence, rhinoceros and Asian elephant milks are rich in medium-chain FAs.[32,35] Horse milk contains lower proportions of C10 : 0 and C12 : 0 (5.41% of total FAs and 7.9% in colostrum, 8.05 and 8.97% 1 mo postpartum), higher proportions of C16 : 0 and C18 : 3n3 (21.3 and 24.1% in colostrum, 23.3 and 20.1% 1 mo postpartum) and similar proportions of C18 : 1n9 and C18 : 2n6 (17.1 and 9.78% in colostrum, 13.7 and 7.5% 1 mo postpartum) compared to the milk of rhino B.[8] The FA profile found in rhino B's samples showed particularly high proportions of medium-chain (C10 : 0, C12 : 0) and unsaturated (C18 : 1n9c, C18 : 2n6c) FAs. These high proportions of medium-chain FAs are not typical in milk of other animal species, apart from the African elephant.[32] The FA profile varied constantly within the lactation period of rhino B, which is not usual.[18] A main cause for this variation is dietary changes.[27] The difference in the diet of rhino B and rhino A over

the year was that they consumed straw, hay, and fresh grass and browse during the summer season while they had only straw, silaged browse, and hay during the winter season. The lack of fresh grass during winter could have led to seasonal differences in the composition of FAs. Grass contains a higher amount of FAs than straw or hay (on average 20%), especially in unsaturated FAs, which could have led to the differences between summer and winter season (Figs. 1, 2).[25] Grass also contains different fat concentrations in different seasons; the concentration is higher in summer than in winter.[33] Furthermore, branches of trees were fed throughout the year. As the leaves contain more fat and unsaturated FAs than the branch itself, it could have accentuated the increase of unsaturated FAs in milk samples in summer compared to winter, since the trees do not carry leaves in winter.[24] Analysis of the straw, hay, and grass that were fed to the two rhinoceros would have been interesting to explain the impact of those diet variations on the FA composition of the milk. However, not only the diet influences the FA profile but also environmental factors which could explain the small deviations found between rhino B and rhino A.[11] Another influencing factor is the stage of lactation, which has been shown in African elephants to affect FA composition.[30]

Lactose is the main component of DM in milk with values between 60.7 and 84.7% (Table 2). It is the main source of energy in milk, but other sugars contribute to the NFE fraction as well. Horse colostrum contains high amounts of oligosaccharides, which would be represented in the NFE fraction, but not in the lactose fraction.[10] It could be that rhinoceros colostrum also contains high concentrations of oligosaccharides, thus having a higher NFE fraction than the lactose concentration in the colostrum. Still, lactose represents the most important source of energy: 67% of the energy content of the milk is provided by lactose (about 12 MJ GE/kg DM). It can quickly be broken down into its two major components, a molecule of glucose and a molecule of galactose, both of which provide readily available energy. The different composition of rhinoceros, horse, and African elephant milks regarding lactose, CP, and EE could reflect the different dietary needs of their offspring. The primary function of rhinoceros and horse milk seems to be the supply of energy with high lactose concentrations. The rhinoceros calves and foals probably have to begin to feed on roughage earlier in life than the African elephant calves to find the proteins and fats they need. Furthermore, a study revealed that Asian elephants have a significant lower digestive efficiency than the horse, while the rhinoceros digestive efficiency is comparable to the horse.[6,7]

DM was lower in rhinoceros and horse milks than in African elephant milk. One explanation is that, due to their high lactose concentrations, rhinoceros and horse milks are more diluted. As shown in a study previously, lactose creates an osmotic effect, thus increasing the milk volume.[1] By contrast, the African elephant concentrates its milk by reducing lactose and increasing fat concentrations.[30]

Ca and Mg decreased over lactation in both rhinoceros and horse milks.[37] This could contribute to push the rhinoceros calves and foals to feed on plant matter earlier in life to ensure their mineral supply. It is quite difficult to find an explanation why Ca and P content increase over the lactation in Asian elephant milk.[1] It may be linked to the fact that elephant calves begin to feed on plants later in life and that their digestion is not as efficient.[6,7] Previous research demonstrated that the energy requirement of elephant calves is mostly covered by the intake of milk, at least during the first 7 mo of life.[1] It seems that milk also is an essential source of protein late in lactation. The natural forage of the free-ranging African elephant generally has a low protein quality, especially in the dry season.[21] This could explain why the CP concentration increases over the lactation period of African elephants. Even if the calf starts foraging, it needs high-quality protein to grow.[21] The high CP and high EE concentration are why African elephant milk contains more energy per gram DM than rhinoceros or horse milk.[29,34]

Furthermore, there is a need to match physiologic stages of lactation rather than simply days of lactation postpartum. African elephant milk 7 mo postpartum should not be considered as mid-lactation milk but as early-lactation milk, if it represents the main source of nutrients for the African elephant calf. In contrast, horse milk 7 mo postpartum may already be considered as late-lactation milk if foals are weaned at 12 mo, and Indian rhinoceros milk 7 mo postpartum should be considered as mid-lactation milk, as weaning occurs within 18 mo. Comparing milk samples collected approximatively 6 mo postpartum showed that the different compositions were probably due to different weaning time points.

Critical reflection of the results and the corresponding analysis should be mentioned. The calibration of the spectrometer was performed on

dairy cow milk (lactose measurement in Suisselab, Zollikofen, Switzerland); the homogeneity of the defrosted whole milk was not always given and some samples were repeatedly frozen. Also, this study is based on two individuals and the knowledge about rhinoceros milk is scarce. As rhino B and rhino A showed similar results, it leads to the conclusion that the analyses are applicable.

CONCLUSION

This study shows that Indian rhinoceros mid-lactation milk has a composition with low EE (3.04% DM basis) and CP (16% DM basis) concentrations and a high lactose concentration (74.6% DM basis). The FA profile shows particularly high proportions of medium-chain FAs. The comparison to other species showed that milk composition of the different rhinoceros species is comparable to that of the horse. Nevertheless, with higher EE concentrations, slightly lower lactose and Mg values, and a different FA profile, horse milk is not an optimal substitute for the rhinoceros calves, unless it is supplemented with a fat source that has a similar FA profile to rhinoceros milk. It is important to improve the knowledge on milk composition of the Indian rhinoceros to create an adequate milk replacer.

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