







Milk composition of white rhinoceros over lactation and comparison with other Perissodactyla

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Funding information

Universiteit van die Vrystaat,
Grant/Award Number: 27241; National Research Foundation, Grant/Award Number: 85939

Abstract

The proximate composition of milk from fifteen free-ranging white rhinoceroses at different lactation stages is reported with detailed analysis of fatty acid composition and minerals. Lactose is the main component at $7.93 \pm 0.53\%$, followed by $0.93 \pm 0.19\%$ protein, $1.76 \pm 0.39\%$ fat, $0.40 \pm 0.18\%$ ash, $0.05 \pm 0.01\%$ glucose, and $0.04 \pm 0.02\%$ non protein nitrogen. The interindividual variation of all the components is high, showing no trend of change over lactation. The K and P content decreased over lactation. The fatty acid composition of rhinoceros milk is characteristic with a high saturated fatty acids content of 62%–84%, of which the medium chain fatty acids form the major portion. The C10:0, C12:0, C16:0, and C18:1c9 are the major fatty acids in the milk fat. The results are compared with the two other Perissodactylae families, the Equidae and Tapiridae. Differences in gross composition are small, but the milk of the Rhinocerotidae have the lowest gross energy, while the milk fats consist of the highest amounts of saturated fatty acids, while the low levels of C16:1c9 and C18:1c9 indicate the lowest mammary $\Delta 9$ -desaturase activity.

KEYWORDS

fatty acid, lactation, lactose, milk, protein, rhinoceros

1 | INTRODUCTION

Pioneering research on milk composition of rhinoceroses was published more than 60 years ago (Aschaffenburg et al., 1961; Grzimek, 1960; Simon, 1959). Analytical techniques were not very advanced then, but with technical advances, updated data is added from time to time. The greatest shortcoming was that many aspects of milk and lactation were assumed to be the same as for humans and domesticated dairy animals. Information on milk from non-dairy mammals showed that certain families and species have different milk composition and also different dynamic changes thereof over the lactation period. It was also learnt that large mammals that offer a suckling time of 2 years or more, such as elephants, display unique changes regarding proximate composition

in general, and oligosaccharides and fatty acids in specific (Abbondanza et al. 2013; Kobeni et al., 2020).

Comparative studies of the milk of different species give insight into biochemical properties of milk synthesis and nutritional properties which would be difficult to investigate in a single species. Detailed information of the composition of milk of commercially exploited animals is available (Casoli et al., 1989; Jenness & Patton, 1976; Voutsinas et al., 1990), as well as for several wild or non-dairy ruminants (Gjostein et al., 2004; Landete-Castillejos et al., 2000; Osthoff et al., 2012; 2017). Of nonruminant herbivores, the horse (*Equus caballus*) (Doreau & Martuzzi, 2006; Markiewicz-Keszycka et al., 2014), ass (*Equus asinus*) (Salimei & Fantuz, 2012; Salimei et al., 2004), zebras (*Equus zebra* and *Equus*

burchellii) (Oftedal & Jenness, 1988), pig (*Sus scrofa*) (Csapo et al., 1996), tapir (*Tapirus terrestris*) (Van Nieuwenhove et al., 2014) and elephant (*Elephas maximus* and *Loxodonta Africana*) (Abbondanza et al., 2013; Kobeni et al., 2020) were also studied in detail.

Regarding the Rhinocerotidae, four studies led to an accumulation of data on the milk of the Indian rhinoceros (*Rhinoceros unicornis*) (Klős et al., 1972; Klős et al., 1974; Nath et al., 1993) and its changes over lactation (Gimmel et al., 2018). Only the proximate and mineral composition, and limited changes over lactation time, were reported for the milk of the black rhinoceros (*Diceros bicornis*) (Gregory et al., 1964), and the proximate composition (Mathews, 1973; Wallach, 1969) as well as minerals, proteins and fat for the white rhinoceros (*Ceratotherium simum*) (Osthoft et al., 2008). The milks of all the rhinoceros species were characterized by a low total solids content of 8.1–16.8%, consisting of 0.2%–1.9% fat, 0.2%–1.2% protein and 5.5%–8.4% lactose. The fatty acid composition of the milk fat of the Indian rhinoceros and white rhinoceros was characterized by a high content of capric acid (10:0), 18%–36% of the total fatty acids, which is more than the 8.4% found in goat milk (Park, 2006), and only surpassed by that of the milk of the elephant, which may contain 35%–70% (Kobeni et al., 2020). The data of Grzimek (1960) on white rhinoceros milk showed nutrient contents in the same order of magnitude as these values, however, Grzimek's data were never taken into account by researchers for species comparison of milk, because the analytical methods were not described (Oftedal, 1984).

Research on energy provision by milk nutrients received great interest (Oftedal, 1984; Power et al., 2002; Tilden & Oftedal, 1997), as did work on the fatty acid content of, and deposition in, milk fat (Dils et al., 1977; Iverson & Oftedal, 1995; Milligan et al., 2007). This aspect is very important, because the very long chain unsaturated fatty acids play a direct role in the neurodevelopment of infants (Kothapalli et al., 2007; Makrides et al., 1994). The fatty acid composition of milk differs between species. In species with foregut fermentation the ingested fatty acids are changed by fermentation. Every species of single stomach digesters, such as the lion (De Waal et al., 2004), domestic cat (Jacobsen et al., 2004), pig (Csapo et al., 1996), African elephant (Osthoft et al., 2012) and various primates (Milligan et al., 2007) have a unique milk fat composition, which may be dictated by a preference for the incorporation of dietary fatty acids or de novo synthesized medium chain (8:0–14:0) fatty acids.

In the mammary gland fatty acid synthesis is terminated by a thioesterase before a chain length of 16 carbons is reached. Depending on the properties of the thioesterase, this termination may be effected after elongation of between 8 and 14 carbons (Neville et al., 1983; Neville, 1998). In general, only ruminant milks contain above 10% of 14:0, which is an indication of the chain length specificity of ruminant thioesterases, but contents above 16% have been found in blackbuck antelope (*Antilope cervicapra*) (Dill et al., 1972), gazelle (*Gazella granti*) (Glass & Jenness, 1971), and members of the Alcelaphinae (Osthoft et al., 2009, 2017). In the Proboscidae (Kobeni et al., 2020, Osthoft et al., 2008) this termination seems to be at 12 carbons or shorter.

Amongst the Perissodactylae, there is evidence that dietary as well as phylogenetic factors may contribute to differences in fatty acid composition of milk fat of the Equidae (Doreau & Martuzzi, 2006; Markiewicz-Keszycza et al., 2014) and the Tapiridae (Van Nieuwenhove et al., 2014). Milk of the equidae contains high proportions of medium length fatty acids (20%–35% C8–C14) and contains very small amounts of both short- and long-chain saturated fatty acids (Salimei & Fantuz, 2012). Equid milk fat contain high levels of α -linolenic acid, which reflects the high level of PUFAs in the diet and the lack of biohydrogenation, as occurs with ruminants.

In the Rhinocerotidae (Gimmel et al., 2018; Klős et al., 1972, 1974; Osthoft et al., 2008) the medium chain fatty acids may be exceeded by the long chain fatty acids (C16 and C18), which may be an indication that the thioesterase allows synthesis of longer chain fatty acids. However, the contribution of long chain fatty acids from the diet may also play a role (Markiewicz-Keszycza et al., 2014).

Insights into the milk composition of three rhinoceros species was obtained by the abovementioned researchers. Similarities and differences were derived from the sparse data. The fact that most studies were performed on single animals, usually without proper description of nutrition, did however complicate comparisons. In the current study we present the first comprehensive data of milk composition of a large number of free ranging white rhinoceroses. Our data from fifteen free-ranging white rhinoceroses, between 0.5 and 18 months lactation, supplement the sparse existing data on rhinoceros milk in general. The composition of white rhinoceros milk over lactation, was not reported before. The data also made it possible to carry out a comprehensive interspecies comparison amongst the Perissodactylae.

2 | MATERIALS AND METHODS

The study complies to the guidelines of the American Society of Mammalogy (Sikes, 2016), the Animals Research Ethics of the University of the Free State (UFS-AED2019/0052), Permission to do research in terms of section 20 of the Animal diseases act, 1984 (Act no. 35 of 1984) of South Africa (permit reference 12/11/1/4), Threatened or Protected Species Regulations (standing permit no. S07969, and registration certificate no. 29414) and Biodiversity Northern Cape Province Integrated Competence (permit no. Fauna 0132/2019).

Milk was obtained from fifteen white rhinoceroses from two semi-extensive wildlife ranches in the Northern Cape province of South Africa. The animals were in good health and roamed on natural vegetation, which was supplemented with lucern during the dry times of 2017–2019. The rhinoceroses were tranquilized for routine veterinarian inspections and pregnancy tests with 6 mg Etorphine, 2500 IU Hyaluronidase and 40 mg Azaperone with a Parmer dart. Reversal was by administration of Diprenorphine. No milk letting agent was administered. Eleven animals between 0.5 and 18 months lactation were sampled during May 2018, and four between 11 and 13 months lactation during May 2019. Milk was drawn by palpation

of the teats while sustained pressure was exerted on the udder. Teats were milked out, producing 5–40 ml milk per teat and milk from each teat was collected separately. Milk was kept on ice (1–2 h while in the field) until freezing facilities were available. Analysis was carried out within 30 days of collection, for which milk was thawed and mixed by swirling in a water bath set to 39°C. Milk from teats were analyzed separately to obtain an average parameter value for each animal.

The water content of approximately 0.5 g white rhinoceros milk was determined gravimetrically after drying in a forced convection drying oven for 2–3 h at 105°C (Association of Official Analytical Chemists, 1990). The dried sample was incinerated at 550°C for two hours, the ash dissolved in 30% nitric acid, and minerals analyzed by inductively coupled plasma optical emission spectroscopy by the Center of Groundwater Studies, University of the Free State (American Public Health Association, 2005). Minerals of twelve animals was analyzed.

Non protein nitrogen (NPN) was fractionated by selective precipitation according to the method of Igarashi (1995). NPN and crude protein (CP) content was determined with a Leco® nitrogen (N) analyzer (LECO Corporation, 2001). The protein content was determined by subtracting NPN from CP and then by multiplying the nitrogen (N) content with a factor of 6.38.

Quantitative extraction of total fat was performed according to Folch et al. (1957) with chloroform and methanol in a ratio of 2:1 (vol/vol). Total extractable fat content was determined by weighing and expressed as g fat/100 g milk. Fatty acids were extracted from the total fat and transesterified to form fatty acid methyl esters (FAME) with 0.5 N NaOH in methanol and 14% boron trifluoride in methanol (Park & Goins, 1994). The FAME were quantified using a Varian 430 gas chromatograph, with a flame ionization detector and a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 µm film thickness). The column temperature was 40–230°C (hold 2 min; 4°C/minute; hold 10 min). The solution of FAME in hexane (1 µl) was injected into the column using a Varian 8400 Autosampler (Varian Inc.) with a split ratio of 100:1. The injection port and detector were both maintained at 250°C. Hydrogen was the carrier gas at 45 psi with nitrogen as makeup gas. The total run time was 59.5 min and chromatograms were recorded using Galaxy Varian Star Chromatography Software (Version 6.41). Nonadecanoic acid (C19:0) was used as internal standard, after it was established that it was not detected in the samples under study. Identification of sample FAME was made by comparing the relative retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component FAME Mix 47885-U together with C18:1c7, C18:2c9t11, C19:0, C22:5). Desaturation indices were calculated as the ratio of monounsaturated fatty acid to the corresponding saturated fatty acid.

Saccharides were extracted from milk with 500 µl 25% trichloroacetic acid per 1 ml milk sample and filtering through Nanosep 3K MF Centrifugal Devices (Pall Life Sciences) in an Eppendorf centrifuge at 13,000 rpm. Samples were de-fatted and de-proteinized by centrifugation at 3000g in Ultrafree-CL (UFC4 LCC 25) filter

devices (Millipore). The filtrate (10 µl) was analyzed with a Waters Breeze High Performance Liquid Chromatography system fitted with Biorad Aminex 42 C (300 × 7.8) mm (Pall Life Sciences) and Waters Sugar Pak 1 (300 × 7.8) mm (Microsep) columns. The analysis was performed at 84°C and analytes were detected using a differential refractive detector. The mobile phase was de-ionized water with a flow rate of 0.6 ml/min. Samples were de-fatted and de-proteinized with Ultrafree-CL (UFC4 LCC 25) filter devices (Millipore, Merck, Johannesburg, South Africa) centrifuged at 3000g. Quantification was done using lactose, glucose and galactose as standards.

The energy content was calculated using factors derived by Perrin (1958) for the energy content of milk fat, carbohydrates and crude protein using the results obtained with the methods described above. The formula to calculate the GE was:

$$\text{GE} = (9.11\text{kcal/g} \times \% \text{Fat} + 5.86\text{kcal/g} \times \% \text{Protein} + 3.95\text{kcal/g} \times \% \text{Carbohydrate}).$$

Individual scatterplots of time into lactation against individual chemical attributes were constructed. Exponential, linear, logarithmic and polynomial regressions were alternately fitted to each graph to determine the best fit (with respect to the R^2 value) between time into lactation and chemical attribute. This was done in an attempt to determine the relationship between time into lactation and chemical attributes (NCSS, 2007).

3 | RESULTS AND DISCUSSION

The average content of the milk components of the white rhinoceroses under study is shown in Table 1. The changes of selected milk components over lactation are depicted in Figure 1–3. With this data on white rhinoceros milk it is now possible to revise the comparison between white-, black- and Indian rhinoceroses as well as with the other families of Perissodactylae, the Equidae, and Tapiridae.

In captivity, mature female white rhinoceroses can breed throughout the year (Metrione & Eyres, 2014). In wild populations, higher birth rates have been recorded during the wet season, correlating with the availability of more resources to support lactation (White et al., 2007). Calves start nibbling grass from an age of two months and will not drink water from natural sources until approximately 5 months old (Owen-Smith, 1975). The average lactation period in the wild is 19 months for female calves and 27 months for males (White et al., 2007). These breeding and nurturing properties of the white rhinoceros, and the fact that all milk samples were collected on a specific date and time of year, implies that the nutrition of all lactating females were exactly the same when milk was collected. Any interindividual variation of milk composition and changes over lactation therefore were independent of the diet.

The total dry matter of the white rhinoceros milk varied between 7.6% and 9.4% with no trend of change over the lactation period. The total dry matter, NPN, ash, protein, lactose and fat content of milk (Table 1) was very similar to that published earlier

TABLE 1 Comparison of the proximate composition and gross energy (average \pm standard deviation) of the milk of white-, black-, and Indian rhinoceroses, lowland tapir, and Equidae species

Nutrient (g/100 g milk)	White rhino	Indian rhino ^a	Black rhino ^b	Horse ^c	Donkey ^c	Plains zebra ^d	Przewalski horse ^d	Lowland tapir ^e
Dry matter	9.37 \pm 0.49	8.47 \pm 0.07	8.82	11.0	9.53	8.3-11.3	10.5	-
Ash	0.40 \pm 0.18	0.30 \pm 0.02	-	0.41	0.41	0.38	0.33	-
Fat	0.93 \pm 0.19	0.30 \pm 0.15	0.20	1.40	0.76	2.20-5.77	1.50	2.47
Protein	1.76 \pm 0.16	1.17 \pm 0.16	1.40	2.14	1.65	1.63-2.15	1.55	6.34
NPN	0.04 \pm 0.02	-	-	-	-	0.06-0.28	-	-
Lactose	7.93 \pm 0.53	6.50 \pm 0.15	6.62	6.1	6.58	6.32-7.00	6.71	5.41
Glucose	0.05 \pm 0.01	--	-	-	-	0.04-0.05	-	-
Gross energy (kCal/100 g)	47.26 \pm 5.12	35.15 \pm 1.05	36.18	48-60	42.06	53.5-80.2	50.19	80.99

Abbreviation: NPN, non protein nitrogen.

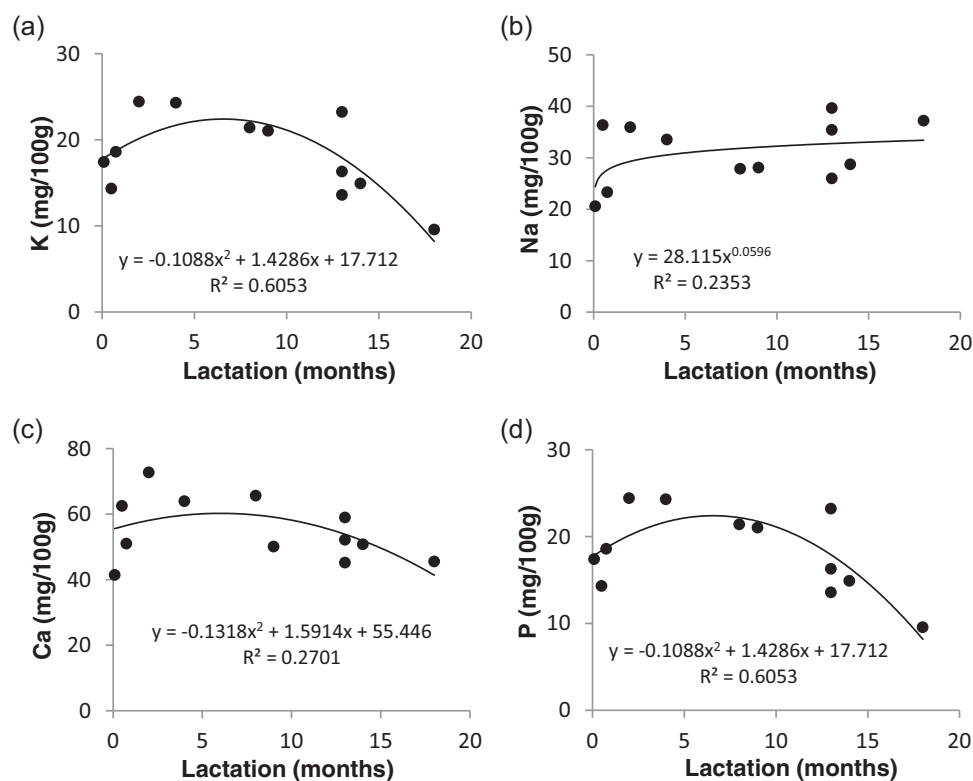
^aGimmel et al. (2018).

^bGregory et al. (1964).

^cSalimei and Fantuz (2012).

^dOftedal and Jenness (1988) and analyses from own archive of six milk samples from three plains zebras.

^eVan Nieuvenhove et al. (2014).

**FIGURE 1** The changes of (A) potassium, (B) sodium, (C) calcium, and (D) phosphorous in white rhinoceros milk over lactation with best-fit trend lines

(Mathews, 1973; Osthoff et al., 2008), and were also similar to that of the black rhinoceros (Gregory et al., 1964; Grzimek, 1960) and Indian rhinoceros (Klös, 1972; 1974; Gimmel et al., 2018). The content of the abovementioned components were also in the same order of magnitude as those reported for the Equidae species, ass (Salimei & Fantuz, 2012), horse (Park, Zhang, et al., 2006; Salimei &

Fantuz, 2012), plains zebra and Przewalski horse (Oftedal & Jenness, 1988), although the plains zebra seemed to have a higher fat content. The milk of the lowland tapir differed substantially, with a higher protein and fat content, but with less lactose. The GE of the white rhinoceros was 47.26 \pm 5.12 kCal/100 g milk, which was 10 kCal higher than that of the Indian (Gimmel et al., 2018) and black

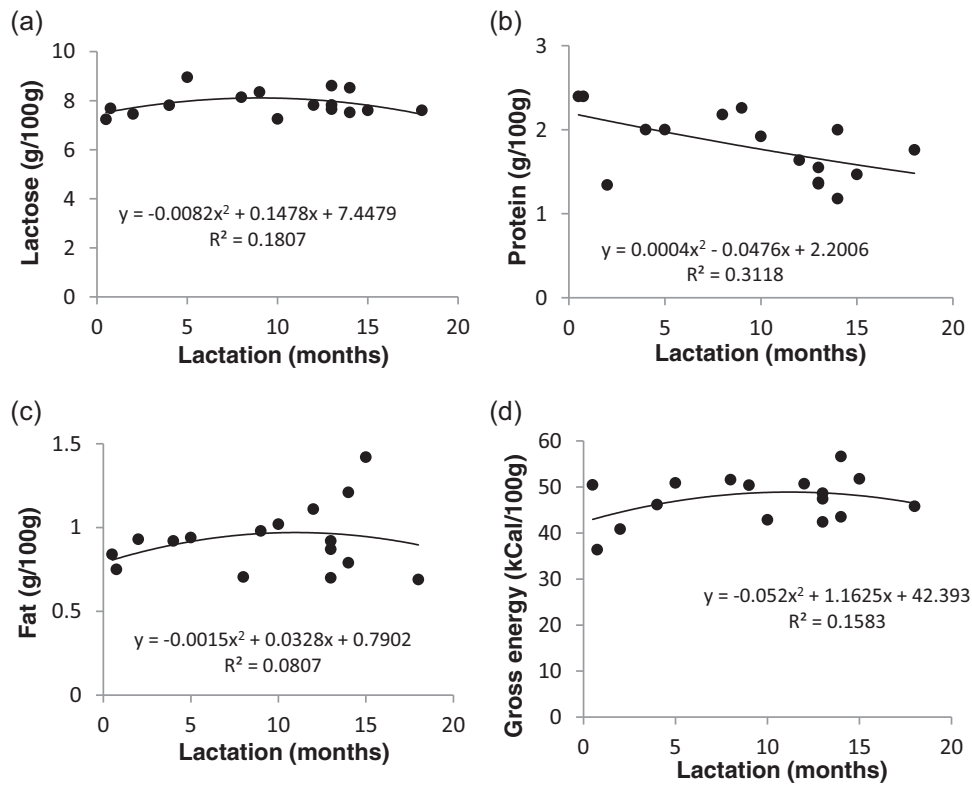


FIGURE 2 The changes of (A) lactose, (B) protein, (C) fat, and (D) gross energy in white rhinoceros milk over lactation with best-fit trend lines

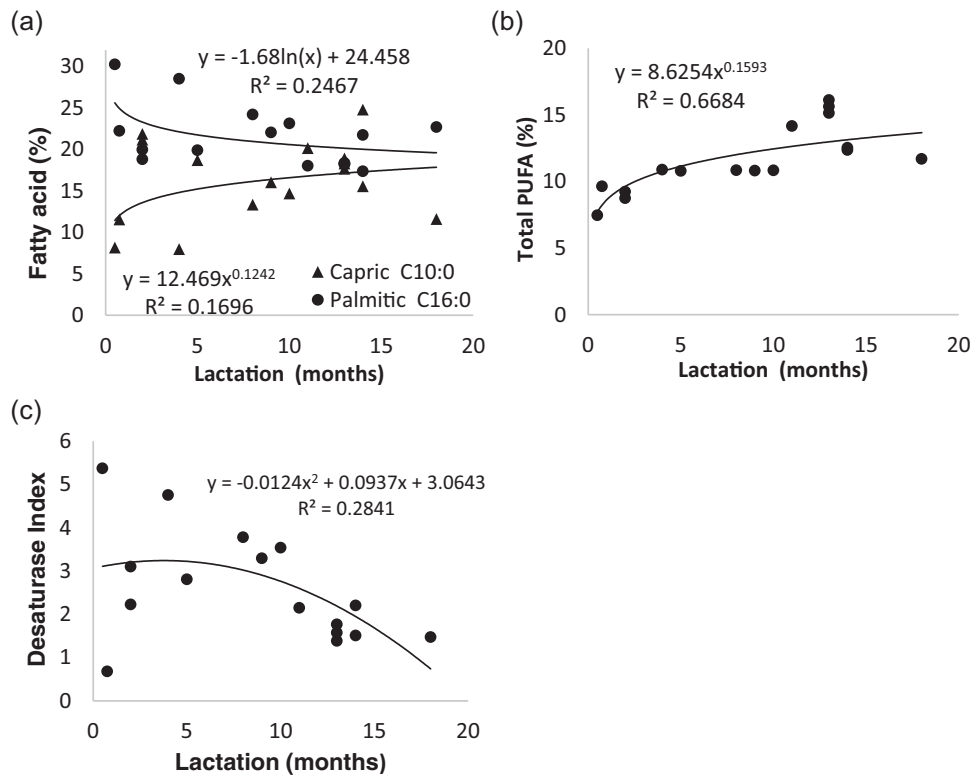


FIGURE 3 The changes of (A) capric and palmitic acid, (B) total PUFA, and (C) C18 desaturase index in white rhinoceros milk over lactation with best-fit trend lines

rhinoceros (Gregory et al., 1964). Available data indicated that the lowland tapir had double the GE (Van Nieuvenhove et al. 2014), with the Equidae displaying levels in-between those of the rhinoceros and tapir.

The ash content of white rhinoceros milk varied between 0.25% and 0.6% (Table 1) with no tendency of change over lactation. This was of the same order as that observed in Indian rhinoceros milk (Gimmel et al., 2018).

The mineral content is shown in Table 2. An interspecies comparison of the K, Na, and Mg of the milk is hampered by lack of data for some of the species. Although there are some differences, the orders of magnitudes are similar, with the possible exclusion of Na. This is different for the trace elements, Cu, Fe, Zn, and Mn, which seem to occur in ten times higher amounts than for the horse and donkey, however Salimei and Fantuz (2012) questioned the data from literature. The K content of white rhinoceros milk increased during the first 4 months of lactation from around 15 mg/100 g milk in early lactation, to around 24 mg/100 g after 2 months and subsequently decreased to below 10 mg/100 g at 18 months (Figure 1a). The milk Na content did not show changes (Figure 1b). Such dynamic changes of K and Na has not been described in the milk of any other rhinoceros.

The Ca:P ratio is approximately 3:1, which is higher than the 1:1 in cow's milk and the 2:1 in horse and human milks (Park, Zhang, et al., 2006). The milk Ca content is comparable with that of the black- (Aschaffenburg, 1961) and Indian rhinoceroses (Gimmel et al., 2018), however, the P content is lower. Within the Perissodactylae, the maximum and minimum Ca and P overlapped. Of the white rhinoceroses under study, the Ca content varied between 41 mg/100 g and above 70 mg/100 g. This curved tendency was, however, not supported by the R^2 of the trend line (Figure 1c). The P increased from

14 mg/100 g to around 22 mg/100 g during the first 4 months of lactation whereafter it decreased to values around 15 mg at 12 months with a further decrease (10 mg/100 g) seen at 18 months (Figure 1d). In horse milk a steady decrease of all minerals was observed over lactation (Park, Zhang, et al., 2006; Salimei & Fantuz, 2012).

The major component of the white rhinoceros milk was lactose. The lactose content varied between 7.23% and 8.4%, but a curved tendency seemed not significant according to the low R^2 of the trend line (Figure 2a). The lactose content, see Table 1, is comparable to that of the Equidae, while that of the lowland tapir seems lower. By comparison, an increase in lactose content from 3.68% in lactose to around 6.7% was reported for Indian rhinoceros, but without any changes over the rest of lactation (Gimmel et al., 2018). In milk of the plains zebra and Przewalski horse (Ofstedal & Jenness, 1988) the lactose content of mature milk was found unaffected by stage of lactation. For the horse and donkey Salimei and Fantuz (2012) reported a similar, unchanged trend, while a tendential increase of milk lactose content was reported throughout lactation by Park, Zhang, et al. (2006) and Doreau and Martin-Rosset (2011).

The glucose content of white rhinoceros milk is similar to that of the plains zebra (Table 1) and followed a similar trend as lactose (data not shown), with a slight increase from 0.04% to 0.05% in early lactation and return to 0.04% (regression line $y = -0.0001x^2 + 0.009x + 0.0411$; $R^2 = 0.3351$).

The milk protein content of the white rhinoceros milk is comparable with that of the Equidae, however, lowland tapir milk contains at least three times that amount (Table 1). In the white rhinoceros milk it decreased over lactation, from approximately 2.4% in the first month of lactation to around 1.8% after 18 months (Figure 2b), however, the R^2 of the trend line was only 0.3118. A decrease in protein content from 8.53% in colostrum to

TABLE 2 Comparison of the mineral composition (average \pm standard deviation) of the milk of white-, black-, and Indian rhinoceroses, and Equidae species

Mineral (mg/100g milk)	White rhino	Indian rhino ^a	Black rhino ^b	Horse ^c	Donkey ^c	Plains zebra ^d	Przewalski horse ^d
Ca	54.99 \pm 9.61	55.5 \pm 6.1	56	50–130	33–114	75 \pm 13.2	82 \pm 15.2
P	18.25 \pm 4.72	34.1 \pm 4.5	40	20–120	32–65	53 \pm 8.9	43 \pm 6.4
Ca/P	3.01	1.63	1.4	1.72	0.93–2.37	1.42	1.91
K	35.31 \pm 10.0	–	86	30–80	24–75	–	–
Mg	6.10 \pm 1.10	7.6 \pm 0.13	–	4–11	4–8.3	–	–
Na	31.05 \pm 6.10	–	37	16.7–20	10–27	–	–
Cu	2.15 \pm 0.59	–	–	0.02–0.10	0.1–0.3	–	–
Fe	0.96 \pm 0.42	–	–	0.02–0.14	0.04–0.26	–	–
Zn	0.33 \pm 0.14	–	–	0.09–0.64	0.12–0.32	–	–
Mn	0.20 \pm 0.02	–	–	0.001–0.005	–	–	–

^aGimmel et al. (2018).

^bAschaffenburg (1961).

^cSalimei and Fantuz (2012).

^dOfstedal and Jenness (1988).

approximately 1.2% in milk was reported for Indian rhinoceros without any changes during the rest of lactation (Gimmel et al., 2018). In milk of the Equidae it was shown that the protein content decreased from colostrum to milk, with no tendency of change over mid- to late lactation (Oftedal & Jenness, 1988; Park, Zhang, et al., 2006; Salimei et al., 2004).

As shown in Table 2, the milk of the plains zebra and lowland tapir have the highest fat content of all the Perissodactylae, while the white rhinoceros milk had the highest fat content of the rhinoceroses. The changes in milk fat content of white rhinoceros milk from 0.84% in the first month of lactation to 1.02% at 9 months was insignificant ($R^2 = 0.0807$) (Figure 2c), while the fat content of Indian rhinoceros milk increased from colostrum up to two months lactation, after which it decreased (Gimmel et al., 2018). A decreasing trend over lactation was reported for horse milk (Doreau & Martin-Rosset, 2011) but not for donkey (Salimei & Fantuz, 2012), plains zebra or Przewalski horse (Oftedal & Jenness, 1988).

Calculation of GE from the three macronutrients showed that the gross energy of white rhinoceros milk was 40–52 kCal/100 g without any trend of change over lactation (Figure 2d). This is higher than the 34–36 kCal/100 g calculated for the mature milk of an Indian rhinoceros (Gimmel et al., 2018), and in the same order as for the horse and donkey (Salimei & Fantuz, 2012). The plains zebra, Przewalski horse (Oftedal & Jenness, 1988) and lowland tapir had GE values between 50 and 80 kCal/100 g, due to the higher fat content.

The average fatty acid composition of white rhinoceros milk is shown in Table 3 together with two examples of extreme differences regarding the content of C10:0, C16:0, and C18:1c9, as well as for the Indian rhinoceros (Gimmel et al., 2018). The data of other Perissodactyla was also included. Unfortunately the same collection of fatty acids was not available for all the species, due to different techniques that were employed.

Nutrition plays an important role in the determination of the fatty acid content of milk fat of monogastric herbivores, as was studied in horse (Hoffman et al., 1998; Markiewicz-Keszycza et al., 2014) and donkey (Martini et al., 2015). These researchers showed that fresh grass and foliage played the greatest role, because they contained more fatty acids than hay, and were responsible for higher amounts of long chain fatty acids in the milk fat. Care therefore has to be taken that differences are not over-emphasized in comparisons.

The C10:0, C12:0, C16:0, and C18:1c9 are the major fatty acids in the milk fat of all the Perissodactylae. The variations in fatty acid composition between individual rhinoceroses mainly involve these. Although C4:0 and/or C6:0 were detected in small amounts in milks of the Equidae and lowland tapir, they were absent in the rhinoceroses. It therefore seems as if absorbance of volatile products produced by fermenting bacteria in the hindgut is not as successful as in the Equidae. Fatty acids longer than 18 carbons were detected at low amounts in the white rhinoceros milk. It is unclear whether they have not been detected, or not been analyzed, in the milk of the Equidae and lowland tapir, or whether they were unavailable in the food.

Taking into account the variations in content of the fatty acids, the milks of the Rhinocerotidae and the Equidae seem to be similar. Although there is a large degree of overlap in maximum and minimum amounts of most fatty acids amongst the species compared here, a few specific differences can be pointed out. The overall highest content of total saturated fatty acids above 60% was observed in rhinoceros milk fat. It seems as if the milk fat of donkey and lowland tapir may in some cases also reach that. This property is therefore not very clear, and maybe affected by nutrition. A C10:0 content higher than 20% was only observed for the rhinoceroses, as was an absence of C14:1c9. A C18:0 content higher than 5% was only observed in milk fats from rhinoceroses and the lowland tapir. A low C18:3c9,12,15 (α -linolenic acid) content was characteristic of the milk of rhinoceroses and tapir. Of odd chain fatty acids, C15:0 was observed in white rhinoceros and lowland tapir, C17:0 only in the former, and C11:0 only in the Indian rhinoceros. A low content of PUFA, specifically C18:2c9,12 (linoleic acid) content, was only observed in the milks of white- and black rhinoceroses. These are essential fatty acids that are obtained from the diet, and may not indicate any species specificity. Low amounts of these two fatty acids have also been reported in milks of the Equidae, as indicated by the minimum values listed in Table 3. These variations are probably due to the differences in the fatty acid composition of diets. Rations richer in forages have more C18:3c9,12,15 than rations rich in concentrates. PUFAs consumed by horses are not microbiologically dehydrogenated before intestinal absorption, therefore milk long-chain fatty acid composition is related to the fatty acid profile of feedstuffs (Salimei & Fantuz, 2012).

The contents of monounsaturated fatty acids give an indication of the mammary $\Delta 9$ desaturase activity for the desaturation of C14:0, C16:0 and C18:0 (Table 3). The absence of C14:1c9 is an indication that the enzyme was not active for C14:0 as substrate in rhinoceros milk, but shows very low activity in the other species. The activity on C16:0 was also low in all the species, but lower in rhinoceroses. On C18:0 the maximum mammary $\Delta 9$ desaturase activity in rhinoceros was approximately 5.0, for lowland tapir 2.5, while the highest activities of between 5 and 30 were calculated for the Equidae.

At first glance, the fatty acid composition of the white rhinoceroses under study seemed to show an increase in the medium chain fatty acids C8:0–C12:0 and a decrease in the long chain fatty acids of C16 to C18 length over lactation. However, with C10:0 and C16:0 as example (Figure 3a) the trend lines show low R^2 values. What is, however, evident from the data in Table 3 and Figure 3a was that great variation in fatty acid types occurred during the first months of lactation, but became stable thereafter. The total SFA content varied between 60% and 83% in the first 6 months and stabilized around 74% thereafter, while the MUFA varied between 6% and 31% and stabilized around 11%. The PUFA showed a trend ($R^2 = 0.6684$) of change from 7.5% in early lactation to 10% at four months and a further increase to an average of 12% (Figure 3b). All three the unsaturated C18 isomers followed this trend. In the current study, a decrease of mammary $\Delta 9$ -desaturase activity for C18:0

TABLE 3 Comparison of the fatty acid composition and desaturase activities (average \pm standard deviation) of the milk of white-, black-, and Indian rhinoceroses, lowland tapir, and Equidae species

FAME (% of total fatty acids)	Species	White rhino (average)	White rhino ^a (ex. 1)	White rhino ^b (ex. 2)	Indian rhino ^c (ex. 1)	Indian rhino ^d (ex. 2)	Black rhino ^e	Horse ^f	Donkey ^f	Plains zebra ^g	Lowland tapir ^h
Abbreviation											
Butyric	C4:0	N.D.	N.D.	N.D.	-	-	-	0.3-0.9	0.32-0.60	-	-
Caproic	C6:0	N.D.	N.D.	N.D.	-	-	-	0.3-1.4	0.28-1.22	0.20-0.28	0.32 \pm 0.15
Caprylic	C8:0	1.75 \pm 0.54	0.76	3.13	7.80	3.63	4.54	0.8-6.1	8.52-12.80	5.01-8.20	2.30 \pm 0.74
Capric	C10:0	17.20 \pm 4.29	7.97	24.81	30.10	9.50	29.86	2.3-16.7	18.65-20.42	7.98-15.56	12.79 \pm 2.65
	C11:0	N.D.	N.D.	N.D.	1.20	1.54	-	-	-	N.D.	-
Lauric	C12:0	10.87 \pm 3.00	3.89	13.48	12.4	6.33	21.35	3.8-14.6	10.67-15.9	5.25-10.82	11.85 \pm 1.39
Myristic	C14:0	5.94 \pm 1.64	4.17	6.03	4.21	2.94	-	4.7-19.2	5.77-10.59	4.18-6.50	4.63 \pm 2.35
Myristoleic	C14:1c9	N.D.	N.D.	N.D.	-	-	-	0.1-2.6	0.22-0.88	0.07-0.21	0.78 \pm 0.14
Pentadecylic	C15:0	7.62 \pm 2.83	6.54	4.19	-	-	-	0.2-0.9	0.32-0.57	0.18-0.50	4.50 \pm 0.67
Palmitic	C16:0	20.52 \pm 3.03	28.52	17.37	7.74	15.3	5.91	12.4-28.5	11.47-29.17	16.30-22.4	11.18 \pm 2.06
Palmitoleic	C16:1c9	1.07 \pm 0.68	2.89	1.39	-	-	-	2.2-9.7	2.37-3.93	1.06-4.20	1.54 \pm 0.35
Margaric	C17:0	4.64 \pm 1.73	3.96	2.50	-	-	-	0.0-1.2	0.22-0.52	0.19-0.30	0.72 \pm 0.11
Heptadecenoic	C17:1c10	0.27 \pm 0.08	0.26	0.29	-	-	-	0.0-1.1	0.27-0.73	0.11-0.22	-
Stearic acid	C18:0	5.20 \pm 0.93	4.79	4.14	2.82	5.05	-	0.3-3.0	1.12-3.91	1.48-2.00	7.30 \pm 2.00
Oleic	C18:1c9	10.67 \pm 3.92	22.75	9.12	12.60	26.4	5.85	9.1-31.6	9.70-22.15	8.15-20.40	17.19 \pm 3.43
Vaccenic	C18:1c7	1.15 \pm 0.51	2.60	1.02	-	-	-	-	-	0.57-1.11	-
Linolelaidic	C18:2t9,12 (n-6)	2.58 \pm 0.98	2.15	1.38	-	-	-	-	-	N.D.	0.11 \pm 0.07
Linoleic	C18:2c9,12 (n-6)	5.36 \pm 1.68	3.93	4.86	18.2	22.6	1.08	3.6-20.3	8.15-15.17	9.68-15.75	17.89 \pm 3.91
α -Linolenic	C18:3c9,12,15 (n-3)	4.86 \pm 2.28	4.49	5.99	2.90	6.62	-	2.2-26.2	6.32-16.33	8.00-20.48	6.67 \pm 1.18
γ -Linolenic	C18:3c6,9,12 (n-6)	N.D.	N.D.	N.D.	-	-	-	-	-	N.D.	0.22 \pm 0.05
Eicosadienoic	C20:2c11,14 (n-6)	0.07 \pm 0.05	0.04	0.05	-	-	-	-	-	N.D.	-
Eicosatrienoic	C20:3c11,14,17 (n-3)	0.22 \pm 0.15	0.22	0.22	-	-	-	-	-	0.17-0.32	-
Arachidonic	C20:4c5,8,11,14 (n-6)	0.04 \pm 0.04	0.05	0.04	-	-	-	-	-	N.D.	-

Fatty acid ratios:

TABLE 3 (Continued)

Species	White rhino (average)	White rhino (ex. 1)	White rhino (ex. 2)	Indian rhino ^c (ex. 1)	Indian rhino ^d (ex. 2)	Black rhino ^e	Horse ^f	Donkey ^f	Plains zebra ^g	Lowland tapir ^h
Saturated fatty acids	73.72 ± 5.93	60.61	69.61	66.27	44.29	61.66	43–49	46.70–67.70	47.41–59.21	55.61 ± 12.06
Mono unsaturated fatty acids	13.15 ± 5.07	28.50	19.58	12.60	26.4	5.85	26.8–36.2	15.30–35.00	10.25–24.65	19.51 ± 3.93
Poly unsaturated fatty acids	13.13 ± 3.25	10.88	10.82	21.10	29.22	1.08	19–20	15.20–30.50	18.00–30.76	25.16 ± 5.41
Omega-6 fatty acids	8.04 ± 1.64	4.71	5.85	18.2	22.6	1.08	3.6–20.3	8.15–15.17	10.10–20.48	18.31 ± 4.10
Omega-3 fatty acids	5.09 ± 2.42	6.17	4.97	2.90	6.62	-	2.2–26.2	6.32–16.33	8.00–15.86	6.67 ± 1.19
Δ9 Desaturase 14 index	0	0	0	-	-	-	0.01–0.02	0.01–0.03	0.02–0.03	0.11 ± 0.08
Δ9 Desaturase 16 index	0.05 ± 0.04	0.10	0.09	-	-	-	0.18–0.34	0.13–0.21	0.06–0.23	0.13 ± 0.09
Δ9 Desaturase 18 index	2.23 ± 0.69	4.75	2.20	4.46	5.22	-	10.5–30.33	5.66–8.66	4.71–13.68	2.35 ± 0.71

Abbreviation: FAME, fatty acid methyl esters.

^aExample of white rhinoceros milk fat with lowest C10:0 and highest C16:0 and C18:1c9 content, Rhino M014 at 4 months lactation.

^bExample of white rhinoceros milk fat with highest C10:0 and lowest C16:0 and C18:1c9 content, Rhino M002 at 14 months lactation.

^cGimmel et al. (2018); Example of white rhinoceros milk fat with highest C10:0 and lowest C16:0 and C18:1c9 content, at 5 months lactation.

^dGimmel et al. (2018); Example of white rhinoceros milk fat with lowest C10:0 and highest C16:0 and C18:1c9 content, at 10 months lactation.

^eGregory et al. (1964).

^fSalimei and Fantuz (2012).

^gUniacke-Lowe & Fox (2011) and analyses from own archive of six milk samples from three plains zebras.

^hVan Nieuvenhove et al. (2014).

over lactation was noted for white rhinoceros (Figure 3c). In early lactation the activity index varied, but after 8 months lactation the index decreased from approximately 3.5 to 1.5, although, the R^2 value is low.

The data of Klös et al. (1972; 1974) of Indian rhinoceros milk showed that the content of fatty acids changed drastically over lactation time from 2.5 months to 15.5 months. However, since no description of the fodder was provided by the author, these changes cannot be ascribed to any factor, such as nutrients or time of lactation.

The fatty acid composition of rhinoceros milk therefore does not show the same tendency of an increase in medium chain fatty acids with a simultaneous decrease of long chain fatty acids, as was observed in milk from another large herbivore, the African elephant (Kobeni et al., 2020). Neither does it show the opposite trend, i.e. a decrease in medium chain fatty acids, as was observed in the lowland tapir (Van Nieuwenhove et al., 2014).

The changes in the milk fatty acid composition of the white rhinoceros, as reported here, is in stark contrast to that observed in the milk of a single Indian rhinoceros over a 13 month period of lactation (Gimmel et al., 2018). Most noteworthy of the latter was that the medium chain fatty acid content was high in early lactation, with C10:0 in excess of 30% and approximately 12% C18:1c9% and 9% C18:2c9,12. These changed to respectively 10%, 25%, and 24% after 10 months lactation. In the white rhinoceros milk analyzed in this study, as well as previously obtained data (Osthoff et al., 2008), the highest amounts of C10:0 observed was 25.5%. Gimmel et al. (2018) noted that for the Indian rhinoceros the winter fodder was barley straw, hay, silage, tree leaves and branches, while in summer, the hay and straw was changed to fresh cut grass as well as fresh leaves and branches. The authors concluded that the summer diet provided a high content of long chain unsaturated fatty acids.

The fatty acid composition of the analyzed white rhinoceros milk was comparable to milk in the 10th month of lactation of the Indian rhinoceros. The milk analyzed here was also collected during the fall, when the availability of field grass became low and supplementation with lucerne was necessary. It therefore seems as if the milk fatty acid composition of rhinoceroses is highly dependent on their diet, similar to that observed in horses (Doreau & Martin-Rosset, 2011; Salimei & Fantuz, 2012).

The fatty acid content of the milk fat of mammals is dependent on the fatty acid content of the diet. In ruminants, other nutritional factors, such as roughage, may also play a role (Park, Zhang, et al., 2006; Salimei et al., 2004). The white rhinoceros is a grazer that prefers short, sprouting green grasses. Depending on availability in terrain and season, *Themeda triandra*, *Panicum maximum*, *Panicum coloratum*, *Cynodon dactylon* and *Urochla mosambicensis* are heavily utilized (Skinner & Smithers, 1990). The rhinoceroses in our study lived within the Savanna Biome in the Eastern Kalahari Bushveld Bioregion known as the Schmidtsdrif Thornveld (SVk 6), Kuruman Mountain Bushveld (SVk 10), and the Olifantshoek Plains Thornveld (SVk 13) as the three main vegetation types described by Mucina and

Rutherford (2006). According to Acocks (1988), the area can be classified as mostly Kalahari Thornveld (Veld Type 17).

With the addition of the current data on white rhinoceros milk, it became possible to revise the comparison with the black- and Indian rhinoceroses, and a high degree of similarity was shown. It was also possible to compare the milk of the Rhinocerotidae with other Perissodactyla. In general, rhinoceros milk contains lower amounts of fat, higher lactose and equal protein compared to the Equidae, while the Tapiridae milk contains the highest amounts of fat and protein, and lowest lactose. Compositional changes in milk of rhinoceroses over lactation are very small compared to that of the Equidae (Salimei & Fantuz, 2012) and Tapiridae (Van Nieuwenhove et al. 2014).

The comparatively large difference between rhino and tapir milk, as opposed to rhino and equid milk, is unexpected when considering evolutionary history. Fossil records indicate a divergence between the suborders Hippomorpha (including Equidae) and Ceratomorpha (including Rhinocerotidae and Tapiridae) more than 50 million years ago (early Eocene period) (Norman & Ashley, 2000). Ceratomorpha diversification that led to the rhinoceroses and tapirs, only followed approximately 45 million years ago. Mitochondrial and nuclear DNA confirmed that the split between Hippomorpha and Ceratomorpha predated divergence between the rhinoceros and tapir lineages (Steiner & Ryder, 2011). Unique milk characteristics within the Tapiridae are thus most likely a secondary development of more recent adaptation.

For immediate application, our data would be useful in the rearing of rhinoceros calves that have been orphaned by poaching. In South Africa alone, the poaching toll has soared from 36 per year between 1990 and 2007 (Orenstein, 2013) to a toll of 1215 in 2014, which decreased to 594 in 2019 (www.helpingrhinos.org). A surrogate milk, that has been used to raise an orphaned white rhinoceros calf successfully, was formula milk powder for horses (Salvana Fohlenmilch, Salvana Tiernahrung GmbH, Elmsdorf, Germany) combined with whey powder (Ludwig et al., 2010) to obtain a fat:protein:lactose ratio of approximately 1:2:4, which is similar to the composition of white rhinoceros milk (Osthoff et al., 2008). Foal formula milk is used successfully in rhinoceros calves for up to 2 years (Personal communication with veterinarian, Jana Pretorius, Rhino Pride Foundation, Bela-Bela, South Africa). Although the natural tolerance of young animals allows for some variation in nutrient composition of milk and surrogate milk, the current results suggest that it might be important to increase the medium chain fatty acid content of formula milk by the addition of coconut or palm kernel oil for their high content of C10:0 (6%), C12:0 (48%), and C14:0 (17%) (Scrimgeour, 2005).

ACKNOWLEDGMENT

The authors would like to sincerely thank to the two game farm owners, who wished to remain anonymous, for allowing them to collect milk during routine management procedures of their white rhinoceros herds. The research was funded by National Research

Foundation (NRF), South Africa [grant number 85939] and University of the Free State [grant number 27241].

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Osthoﬀ, G., Beukes, B., Steyn, A. C., Hugo, A., Deacon, F., Butler, H. J. B., O'Neill, F. H., & Grobler, J. P. (2021). Milk composition of white rhinoceros over lactation and comparison with other Perissodactyla. *Zoo Biology*, 1–12. <https://doi.org/10.1002/zoo.21618>