MILK CHARACTERISTICS OF A CAPTIVE INDIAN RHINOCEROS (*RHINOCEROS UNICORNIS*)

Nabin C. Nath, M.V.Sc., Ph.D., Abedul Hussain, G.V.Sc., and Faridur Rahman, M.V.Sc.

Abstract: The physicochemical characteristics of the milk of a captive Indian rhinoceros (*Rhinoceros unicornis*) were determined at 30, 37, and 44 days postpartum. The milk was ivory white and aromatic. The mean value for pH was 6.49 and specific gravity was 1.0296. The mean values of different components in whole milk in g/dl were as follows: total solids, 9.81; fat, 1.40; nonfat solids, 8.41; lactose (total reducing sugars), 7.60; total protein, 1.39; casein, 1.00; whey protein, 0.39; beta-lactoglobulin, 0.23; alpha-lactalbumin, 0.17; serum albumin, 0; immunoglobulin, 0; urea, 0.04; calcium, 0.08; sodium, 0.02; potassium, 0.09; inorganic phosphorus, 0.02; and chloride, 0.04. Values for total protein, casein, whey protein, sugar, and minerals in the present investigation were similar to values reported previously in Indian rhinoceros milk, but not in milk of the white rhinoceros (*Ceratotherium simum*) or black rhinoceros (*Diceros bicornis*). Comparisons of the present values with reported values in milk of domestic horses, elephants (*Elephas maximus*), cows, buffalo (*Bubalis bubalis*), goats, and sheep revealed similarity only with milk of the horse.

Key words: Indian rhinoceros, Rhinoceros unicornis, milk composition.

INTRODUCTION

Knowledge of milk composition is a prerequisite for preparing an appropriate milk substitute for a species. The State of Assam in northeastern India experiences heavy flooding every year that causes separation of Indian rhinoceros (Rhinoceros unicornis) calves from their mothers. As a result, zoo veterinarians must rear these orphaned calves on artificial milk. Limited information is available on composition of rhinoceros milk. The chemical composition and vitamin concentrations of a milk sample collected at 19 mo postpartum from an African black rhinoceros (Diceros bicornis)² and chemical composition of samples collected at 5 and 18 mo postpartum from a white rhinoceros (Ceratotherium simum)14 have been reported. The chemical composition of colostrum from a captive white rhinoceros¹⁰ and African black rhinoceroses⁶ and changes in composition during lactation in the latter also have been reported.⁶ Major milk constituents and different mineral concentrations in 10-, 41-, and 53-day postpartum milk of an Indian rhinoceros have also been reported.8 A comparative review of milk composition of rhinoceroses and other species has been compiled by Oftedal.¹¹ Small numbers of samples, inattention to lactation stage, potential sampling bias, inappropriate analytical procedures, and/or methodological difficulties plague many studies of lactation in wild animals. A more reliable data base is needed both for comparative purposes and for use in formulation of artificial milk for neonatal mammals.¹¹ In this communication, data on the milk composition of an Indian rhinoceros are reported.

MATERIALS AND METHODS

A male rhinoceros calf was born at the State Zoo cum Botanical Garden, Assam, India on 30 March 1989. The dam of the calf was zoo born. The sire was wild-caught at the age of 4 yr. The rhinoceros bull and cow were 13 and 11 yr old, respectively. The rhinoceros cow was mated with the bull on 11 November 1987, and this was her second calf.

The calf suckled the mother generally at 15–20-min intervals. During the day the maximum suckling interval was 1 hr from

From the Department of Animal Physiology, College of Veterinary Science, Assam Agricultural University, Guwahati-781022, Assam, India (Nath); and the State Zoo cum Botanical Garden, Assam, Guwahati-781005, Assam, India (Hussain, Rahman).

0400 hr to 0500 hr, at which time the mother left the calf in the shed and came out to wallow and eat. At about 0500 hr the calf joined the mother for suckling. The rhinoceros cow allowed manipulation of its udder by the attendant. About 30 ml of milk was manually collected at 0500 hr on 30, 37, and 44 days postpartum before suckling by the calf. Before collecting the milk, the udder was washed thoroughly with distilled water.

The pH of the milk was determined with a digital pH meter (Digital pH meter 335, Systronics, Ahmedabad, India). The specific gravity (pycnometer), total solids (gravimetric), and fat (Gerber) were determined by standard methods.1 Total solids were determined after heating 5 ml of milk in a hotair oven at 98 \pm 1°C for 4 hr, and a constant weight was obtained 20 min thereafter. Differences between total solids and fat concentration were considered to be nonfat solids. Because milk contains no significant amount of reducing sugars other than lactose,¹¹ the total reducing sugars were considered to be lactose. Lactose (Folin and Wu)¹² was estimated by Benedict reactions based on reduction of cupric ion to cuprous ion by lactose in alkaline media, with subsequent combination with phosphomolybdate to produce colored compounds. Anhydrous alpha-D-lactose was used as the standard. Estimation of urea,3 calcium,15 and inorganic phosphorus (Fiske and Subbarow)12 were determined spectrophotometrically (Spectrophotometer, Bausch and Lomb, 820 Linden Ave., Rochester, New York 14610). Chloride (Schales and Schales)¹² was estimated by titrating against mercuric nitrate using diphenylcarbazone as the indicator. Sodium and potassium were estimated¹² by flame photometry (Mediflame 127, Systronics, Ahmedabad, India). Proteins (total and whey) were estimated by the phenol-folin-Ciocalteau method⁹ based on the reaction of the phenol groups of tryptophan and tyrosine in milk proteins with the reagent to form a blue color. Bovine serum albumin was used as the standard.

Isoelectric precipitation of casein at 40°C was accomplished by adjusting the pH of the milk to 4.6 with acetic acid and sodium acetate. Differences between total protein and whey protein were used to estimate casein concentration. Polyacrylamide gel electrophoretic fractionation of whey proteins was performed by the method of Davis,⁴ as described for buffalo milk.¹³

RESULTS AND DISCUSSION

The gestation length for this female was 506 days, which is longer than the 480–482 days published for the Indian rhinoceros.⁵ The height, girth, and body length of the calf were 66, 97, and 105 cm, respectively.

The short suckling interval of the Indian rhinoceros calf was similar to that of the horse foal, which suckles several times per hour.¹¹ The lactation length of the cow was 20 mo. A lactation period of more than 19 mo has been reported for the African black rhinoceros.² To minimize sampling bias. several precautions were taken as suggested by Oftedal.¹¹ It was not possible to separate the mother from her calf; therefore, the maximum normal suckling interval was used to collect each milk sample (i.e., 80 min from the previous suckling interval). It has been suggested that sampling bias may not be a concern for the black rhinoceros because milk fat concentration does not change markedly during the course of milking in this species.⁶

The milk of the Indian rhinoceros was ivory white and aromatic. The appearance of 19-mo lactation milk in the African black rhinoceros² was reported to be white and watery. Lactation stages of the Indian rhinoceros have not yet been established, but milk collections 30–330 days postpartum from the black rhinoceros¹¹ were considered midlactation samples. On the above basis, milk collected in the present study at 30, 37, and 44 days postpartum from the Indian rhinoceros were considered midlactation samples (Table 1). The major constituents in milk from this Indian rhinoceros were compared (on a whole and dry matter basis)

	F	ostparturient day			
	30 37		44	Mean ± SE	
pH	6.47	6.51	6.48	6.49 ± 0.01	
Specific gravity	1.0293	1.0300	1.0295	1.0296 ± 0.0002	
Total solids (g/dl)	9.64	9.88	9.92	9.81 ± 0.09	
Fat (g/dl)	1.50	1.40	1.30	$1.40 \pm 0.06 \ (14.27)^{a}$	
Nonfat solids (g/dl)	8.14	8.48	8.62	8.41 ± 0.14 (85.73)	
Lactose (g/dl)	7.21	7.80	7.80	7.60 ± 0.02 (77.47)	
Total protein (g/dl)	1.44	1.37	1.37	$1.39 \pm 0.02 (14.17)$	
Casein (g/dl)	1.00	0.97	1.03	1.00 ± 0.02 (10.19)	
Whey protein (g/dl)	0.44	0.40	0.34	0.39 ± 0.03 (3.98)	
Beta-lactoglobulin (mg/dl)	264.00	232.00	187.00	227.67 ± 22.33 (2.31)	
Alpha-lactalbumin (mg/dl)	176.00	168.00	153.00	165.67 ± 6.74 (1.68)	
Serum albumin	ND^{b}	ND	ND		
Immunoglobulin	ND	ND	ND		
Urea (mg/dl)	40.50	48.00	43.80	44.10 ± 2.17 (0.45)	
Calcium (mg/dl)	80.16	82.16	90.18	84.17 ± 3.06 (0.87)	
Sodium (mg/dl)	23.00	25.60	25.60	$24.70 \pm 0.87 (0.24)$	
Potassium (mg/dl)	90.16	92.14	90.16	$90.82 \pm 0.66 (0.92)$	
Inorganic phosphorus (mg/dl)	24.44	24.44	26.69	$25.19 \pm 0.75(0.25)$	
Chloride (mg/dl)	36.50	35.50	37.50	$36.50 \pm 0.58 (0.37)$	

Table 1. Chemical composition of milk of an Indian rhinoceros (Rhinoceros unicornis) at midlactation.

^a Values in parentheses = % on a dry matter basis.

^b ND = not detectable.

with early-lactation, midlactation, and latelactation milk from other rhinoceroses as well as with midlactation milk from some domestic animals (Table 2).

The pH of the Indian rhinoceros milk in the present study (6.5) was found to be similar to that reported for 18-mo postpartum milk of the white rhinoceros (6.4)¹⁴ and the midlactation milk of the cow (6.6 \pm 0.10).¹² The specific gravity of the Indian rhinoceros milk (1.0296) was also similar to that of midlactation milk of the horse (1.0347), elephant (*Elephas maximus*) (1.0313), cow (1.0313), buffalo (*Bubalis bubalis*) (1.030), and goat (1.0305).¹

Differences observed in major milk constituents of the Indian rhinoceros in this study, compared with those for early-lactation and late-lactation rhinoceros milk (Table 2) might be due to differences in stage of lactation which exert an influence on milk composition in all species.^{1,7,11-13} Perissodactyl colostrum was typically high in total solids and protein, but was low in sugar. Fat concentration was either high, low, or unchanged relative to midlactation milk.^{6,11} Lower sugar and similar protein concentrations in the milk of the black rhinoceros at late-lactation were also reported.⁶

To obtain appropriate comparative data as suggested by Oftedal,11 the major midlactation milk constituents of the Indian rhinoceros in this study (Table 1) were compared with midlactation milk of other rhinoceroses and domestic animals (Table 2). The lower fat concentrations found in milk in the present study, compared with a previous report for the Indian rhinoceros (where the analytical method was not mentioned),8 might be due to the use of different analytical methods.¹¹ The higher fat concentrations found in Indian rhinoceros milk as compared to that of the black rhinoceros might be due to species differences.^{1,7,11,12} Although the protein concentration was the same, Indian rhinoceros milk was much higher in fat and lower in lactose than that of the black rhinoceros, which may have been related to the higher level of total solids in the Indian rhinoceros milk. The major

Lactation stage/species	Days postpartum	No. of samples	Total solids (%)	Fat (%)	Total protein (N × 6.38) (%)	Sugar (%)	Ash (%)	Ref- er- ence
Early lactation								
White rhinoceros (Ceratotherium simum cottoni)	1	1	14.23 +ª	1.73 (12) ^a =	7.34 (52) +	NR♭	NR	10
Midlactation								
Indian rhinoceros (Rhinoceros unicornis)	41	1	NR	4.00 +	1.32	8.30 =	NR	8
White rhinoceros (C. s. simum)	150	1	8.84 _	0.60 (7)	1.54 (17) =	6.50 (74)	0.2 (2)	14
Black rhinoceros (Diceros bicornis)	30-330	11	8.8	0.2 (2)	1.4 (16) =	6.6 (75)	0.3 (3)	11
Horse (Equus caballus)	24–54	25	10.5 =	1.3 (12)	1.9 (18) =	6.9 (66) =	0.4 (4)	11
Asian elephant (Elephas maximus)	62–120	3	17.7 +	7.3 (41)	4.5 (24)	5.2 (29)	0.6 (3)	11
Cow (Bos taurus)	Mature	2,000	12.4 +	3.7 (30)	3.2 (26)	4.6 (37)	0.7 (6)	11
Water buffalo (Bubalis bubalis)	30	42	16.8 +	6.5 (39) +	4.3 (24)	4.9 (29)	0.8 (5)	11
Goat (Capra hircus)	14-56	120	12 +	3.8 (32)	2.9 (22)	4.7 (39)	0.8 (7)	11
Sheep (Ovis aries)	13-35	20	18.2 +	7.3 (39) +	4.1 (23)	5.0 (27)	0.8 (4)	11
Late lactation								
White rhinoceros (C. s. simum)	540	1	8.26 _	trace	1.18 (14) =	6.85 (83)	0.23 (3)	14
Black rhinoceros (Diceros bicornis)	570	1	8.10 -	trace	1.54 (19) =	6.06 (75)	0.34 (4)	2

Table 2. Comparative data on major milk constituents of some species of animals with midlactation milk of the Indian rhinoceros in the present study.

^a Values in parentheses = % on a dry matter basis.

^b NR = not reported.

 $^{\rm c}$ The signs =, +, or - indicate that the concentration of the milk constituent is similar to, higher, or lower, respectively, than the concentration in midlactation milk of the Indian rhinoceros in this study.

midlactation milk constituent concentrations of the Indian rhinoceros were similar to those of the horse, but Indian rhinoceros milk was higher in sugar and lower in total solids, fat, and protein concentration as compared to milk of the elephant, cow, buffalo, goat, and sheep (Table 2). Species differences in major milk constituents have been reported by others.^{1,7,11,12} The horse and rhinoceros secrete milk that is very low in dry matter concentration, ranging from 9 to 11%, with sugar constituting the major portion (59–75%) and fat the minor portion (2– 15%).¹¹ Information on minor milk constituents at midlactation of different species and comparative data are not available. Therefore, only qualitative comparisons of the estimated minor milk constituents in the present study with other species of rhinoceros were made. Of the total protein in Indian rhinoceros milk, 72% was casein, which is similar to that of the black rhinoceros $(1.11 \text{ g/dl})^2$ and white rhinoceros $(0.91 \text{ g/dl})^{.14}$ Casein also contributed the major protein in cow (76-84%),^{7,11,12} buffalo (79%),¹³ and goat $(75\%)^{12}$ milk. Polyacrylamide gel electrophoresis (Fig. 1) revealed

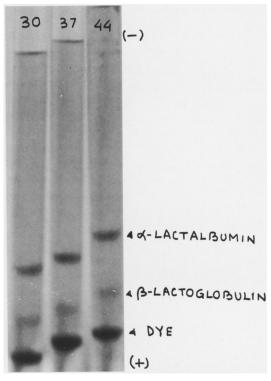


Figure 1. Polyacrylamide gel (7%) electrophoretic pattern (pH 8.5) of 30-, 37-, and 44-day postpartum whey protein of Indian rhinoceros milk.

that the midlactation whey protein of Indian rhinoceros milk contained two protein components, beta lactoglobulin (58%) and alpha lactalbumin (42%). Serum albumin and immunoglobulin fractions were not found. The urea concentration was similar to that in cow's milk $(32 \pm 9.3 \text{ mg/dl})$.³ The reported values for non-protein nitrogen concentration in 19-mo postpartum milk of a black rhinoceros² and 41-day postpartum milk of an Indian rhinoceros⁸ were 20 mg/ dl and 38 mg/dl, respectively. Mineral concentrations in the present study of Indian rhinoceros milk were similar to those reported for 41-day postpartum milk of an Indian rhinoceros.8

CONCLUSION

Concentrations of the major milk constituents in Indian rhinoceros milk were similar to those of the horse, but differed from milk of elephants, cows, buffaloes, goats, and sheep, in which the milk was higher in protein and fat and lower in lactose. From the present findings, it is concluded that whole unmodified cow, buffalo, goat, and sheep milk probably are not suitable for feeding Indian rhinoceros calves. However, there is a need for further detailed studies on the colostrum and milk of rhinoceroses to determine the immunoglobulin, vitamin, free amino acid, fatty acid, and mineral concentrations.

Acknowledgments: We are thankful to Saniram, the attendant of the Indian rhinoceros of State Zoo cum Botanical Garden, Assam, for collecting the milk. We are grateful to the Dean of the College of Veterinary Science, Guwahati, Assam, India for providing the facilities for this work.

LITERATURE CITED

1. Aggarwala, A. C., and S. D. Ahmad (eds.). 1940. A Laboratory Manual of Milk Inspection, 2nd ed. Messrs Gulab Chand Kapur and Sons, Lahore, India. Pp. 3–82.

2. Aschaffenburg, R., M. E. Gregory, S. J. Rowland, S. Y. Thompson, and V. M. Kon. 1961. The composition of the milk of the African black rhinoceros (*Diceros bicornis*; Linn.). Proc. Zool. Soc. Lond. 137: 475–479.

3. Bhavadasan, M. K., V. S. Rajput, and N. C. Ganguli. 1982. A simple colorimetric method for the determination of urea in milk. Indian J. Dairy Sci. 35: 263–266.

4. Davis, B. J. 1964. Disc electrophoresis-II. Methods and application to human serum proteins. Ann. N.Y. Acad. Sci. 121: 404-427.

5. Gariola, M. K. 1987. Study of parturition in great Indian rhinoceros. Indian Vet. Med. J. 11: 185–190.

6. Gregory, M. E., S. J. Rowland, S. Y. Thompson, and V. M. Kon. 1965. Changes during lactation in the composition of the milk of the African black rhinoceros (*Diceros bicornis*). Proc. Zool. Soc. Lond. 145: 327–333.

7. Jenness, R., and S. Patton (eds.). 1969. Principles of Dairy Chemistry. Wiley Eastern Private Limited, New Delhi, India. Pp. 1–29.

8. Lang, E. M. 1961. Beobachtungen am Indischen Panzernashorn (*Rhinoceros unicornis*). Der Zoolog. Garten 25: 369–409.

9. Lowry, O. H., N. J. Rossenberg, A. F. Lewis, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275. 10. Mathews, M. 1973. Birth of a white rhinoceros in captivity. J. Zoo Anim. Med. 4: 18.

11. Oftedal, O. T. 1984. Milk composition, milk yield and energy output at peak lactation: a comparative review. Symp. Zool. Soc. Lond. 51: 35-85.

12. Oser, B. L. (ed.). 1979. Hawk's Physiological Chemistry, 4th ed. Tata McGraw-Hill, New Delhi, India. Pp. 368–393.

13. Singh, L. N., N. C. Nath, A. Kumar, P. L. Yadava, and H. S. Pandey. 1982. A full lactation study on the milk protein profile and casein composition in domestic water buffalo. Indian J. Dairy Sci. 35: 570-574.

14. Wallach, J. D. 1969. Hand-rearing and observations of a white rhinoceros (*Diceros s. simus*). Int. Zoo Yrbk. 9: 103–104.

15. Webster, W. W., Jr. 1962. A simple microspectrophotometric method for determination of serum calcium. Am. J. Clin. Pathol. 37: 330–333.

Received for publication 1 July 1991.