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ARTICLE

Characterization of gross composition, energy value, and fatty acid profile of milk from lowland tapir (*Tapirus terrestris*) during different lactation periods

C. Van Nieuwenhove, M.E. Pérez, M. Hernández, and P. Luna Pizarro

Abstract: We evaluated gross composition, energy value, and fatty acid profile of colostrum, transitional, and mature milk of lowland tapir (*Tapirus terrestris* (L., 1758)) from Argentina. Samples were obtained from six healthy animals kept in captive or semicaptive conditions. Protein content varied over time from 6 to 19 g/100 g of milk; carbohydrates varied from 2.4 to 5.4 g/100 g of milk; fat varied from 2.4 to 17.3 g/100 g of milk. Energy value also varied over time, with colostrum having the highest value. Fatty acid profile revealed a unique pattern in tapir milk, characterized by the presence of higher amounts of lauric, myristic, and pentadecanoic acids than in milk from other equids. Traces of conjugated linoleic acid (CLA) were measured, being the only *trans* fatty acid detected in tapir milk. Neither butyric acid (C4:0) nor fatty acids longer than C18 were found in tapir milk. The characteristics of tapir milk include high concentration of fat, caseins, and whey proteins. Gross composition and saturated fatty acid to unsaturated fatty acid (SFA/UFA) ratio of tapir milk were more similar to that of horse milk than rhinoceros milk. The present study sheds light on tapir fatty acid metabolism and on nutritional requirements of their newborns, which can be used to improve conservation and management strategies. Furthermore, different periods of lactation were evaluated in this species for the first time, thus contributing to the general knowledge of milk from other members of the order Perissodactyla.

Key words: Tapirus terrestris, lowland tapir, endangered species, milk, fatty acid profile, energy value.

Résumé : Nous avons évalué la composition brute, la valeur énergétique et le profil des acides gras du colostrum, du lait de transition et du lait mature de tapirs terrestres (*Tapirus terrestris* (L., 1758)) d'Argentine. Des échantillons ont été obtenus de six animaux en santé gardés dans des conditions de captivité ou de semi-captivité. La concentration de protéines variait dans le temps de 6 à 19 g/100 g de lait, celle de glucides, de 2,4 à 5,4 g/100 g de lait, et celle de lipides, de 2,4 à 17,3 g/100 g de lait. La valeur énergétique variait également dans le temps, le colostrum présentant la valeur la plus élevée. Le profil des acides gras du lait de tapir présentait une distribution singulière caractérisée par des quantités plus importantes des acides laurique, myristique et pentadécanoïque que celles du lait d'autres équidés. Des traces d'acide linoléique conjugué (ALC) ont été mesurées, ce dernier étant le seul acide gras *trans* détecté dans le lait de tapir. L'acide butyrique (C4:0) et des acides gras plus longs que C18 n'ont pas été détectés. Les caractéristiques du lait de tapir comprennent de fortes concentrations de lipides, de caséines et de protéines de lactosérum. La composition brute et le rapport des acides gras saturés et des acides gras non saturés (AGS/AGN) du lait de tapir ressemblaient plus à ceux du lait de jument qu'à ceux du lait de rhinocéros. L'étude jette un nouvel éclairage sur le métabolisme des acides gras chez les tapirs et sur les besoins nutritifs de leurs nouveau-nés qui peut server à améliorer les stratégies de conservation et de gestion. De plus, différentes périodes de lactation ont été évaluées pour la première fois chez cette espèce, ce qui contribue également à accroître les connaissances générales sur le lait d'autres membres de l'ordre des périssodactyles. [Traduit par la Rédaction]

Mots-clés : Tapirus terrestris, tapir terrestre, espèce en voie de disparition, lait, profil des acides gras, valeur énergétique.

Introduction

Milk represents a highly complex food produced by mammary glands that supply all the nutritional requirements of newborns, thus why its composition varies among different mammals. Lactogenesis occurs together with parturition, although small amounts of precolostrum could be formed during the later stages of pregnancy. Different macro- and micro-elements present in milk act as essential compounds for the nourishment of the newborn, depending on the biology of each species. As result, mammals of related groups present similar gross composition of milk, although some exceptions do occur. Milk from ruminants and humans are the most studied; among the order Perissodactyla, which includes the families Equidae, Tapiridae, and Rhinocerotidae (Nowak and Paradisio 1983), only milk from horses and donkeys have been extensively examined.

The genus *Tapirus* Brisson, 1762 has four living species, one distributed in Asia and three in Central and South Americas (García et al. 2012), and are closely related to horses and rhinoceroses. Tapirs are exclusively herbivorous–frugivorous (Richard and Juliá 2000), grazing on different types of food. Under captive or semicaptive condition, a balanced diet requires that supplements be incorporated in their diet; the fermentation process occurs in the caecum, while absorption occurs in the caecum and colon (Janis 1976). The gestation period is around 13 months with usually

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one calf being born. After birth, the calf follows the mother and nurses at regular intervals between 3–4 h, which result in a mass gain of approximately 0.6 kg/day (Mallinson 1969); their mass doubles after 14 or 15 days. Knowing the gross composition of milk from wild animals is a key factor in their care and management, which allows for the manipulation of different feeding strategies to ensure the survival of the newborn. Data regarding tapir milk composition are almost nonexistent. In Argentina, lowland tapirs (*Tapirus terrestris* (L., 1758)) are the only natural inhabitants of the lowlands (Padilla and Dowler 1994; Soler 2006; Chalukian 2008; Chalukian et al. 2013); they are also found in different parks and ecological reserves. They are in danger of being extinct (Díaz and Ojeda 2000).

Different milk compounds vary over time, including fatty acids. In general, colostrum consists of high protein and total solids and low carbohydrates compared with mature milk. However, fat content could be high, low, or unchanged during lactation. This was reported by Oftedal (1984), who described the milk composition of at least 194 species, as well as the variation in milk components during early (colostrum), middle (transitional), and late (mature) lactations. To the best of our knowledge, few studies have described the composition of milk from tapirs; these studies have used no more than two females (Ormrod 1967; Toyoda et al. 1970; Pérez et al. 2010), and only one of them has evaluated the fatty acid profile of tapir milk (Toyoda et al. 1970). In one additional study, Oftedal and Iverson (1995) included the general composition of tapir milk based on unpublished data and personal communications. Components of tapir milk have been reported in colostrum and mature milk; however, to date, the composition of transitional milk is still unknown.

Our study aims to evaluate the gross composition, energy value, and fatty acid profile of milk from lowland tapirs from Argentina and their variation over time. Additionally, we compared the composition of milk from tapirs with the composition of milk from other perissodactyls and elephants to improve our knowledge of closely related mammals.

Materials and methods

Milk samples

Samples of milk from six healthy lactating female lowland tapirs aged 3–10 years were obtained for this study. Three semicaptive animals were from the Reserva Experimental Horco Molle of the Universidad Nacional de Tucumán (Yerba Buena; 26°38′S to 26°57′S, 65°26′W to 65°20′W), two captive animals were from the Temaikén park (Buenos Aires; 34°21′50″S, 58°48′10″W), and one captive tapir was from the Estación de Fauna Autóctona “Finca Las Costas” (San Lorenzo, Salta; 24°43′00″S, 65°29′00″W). Milk samples were handled collected from both teats and aliquots were pooled into plastic flasks without any preservatives. Neither milk-letting agent nor drug tranquilizer was administered to animals. The final volume collected varied from 10 to 15 mL. Samples were collected between 2005 and 2013, during parturition of animals. Aliquots of individual samples were immediately frozen at −20 °C until their analysis; samples were processed within the first month of their collection.

Sequential milk samples were obtained from three animals (one captive and two semicaptive animals) at 0–5, 7–12, and 17–25 days and were classified as colostrum (early lactation), transitional milk (mid-lactation), and mature milk (late lactation), respectively. In other tapirs, samples were taken during early and late lactation (i.e., colostrum and mature milk, respectively). Tapirs in captivity were feed commercial chow supplemented with fruits and ad libitum water, while tapirs in semicaptivity (only those in Reserva Experimental Horco Molle) were feed commercial chow supplemented with fruits and ad libitum water and allowed to graze naturally.

Chemical reagents

All pure-grade (99%) reagents were purchased from Sigma–Aldrich (St. Louis, Missouri, USA). High performance liquid chromatography grade solvents were used for fatty acid extraction and derivatization.

Milk composition

Total and whey proteins of milk were determined according to Lowry et al. (1951), using seroalbumin as a standard. Casein content was calculated as the differences between total and whey proteins. Carbohydrates were measured according to Winzler's (1955) procedure. Fat was determined according to Folch's (1957) procedure, which is explained below. Gross energy was determined using Perrin's (1958) formula, with coefficients of 9.11, 5.86, and 3.95 for the percentage of fat, protein, and lactose, respectively. pH was measured with a Metrohm pH meter (model 692; Metrohm, Herisau, Switzerland).

Fatty acid analysis

Fatty acid profile was analyzed according to the method described in Van Nieuwenhove et al. (2011). Lipids were first extracted following Folch's (1957) procedure, with a chloroform–methanol mixture (2:1 *v*/*v*). Lipids were hydrolyzed with 3 mL of 0.9% (*m*/*v*) NaOH in methanol at 100 °C for 15 min. Fatty acids were methylated with 4% (*v*/*v*) HCl in methanol at 60 °C for 20 min, then extracted with hexane and evaporated under a nitrogen stream. Fatty acid methyl esters were dissolved in hexane and then injected into a gas chromatograph (model 6890N; Agilent Technologies, Palo Alto, California, USA) equipped with a flame ionization detector and automatic injector (model 7683; Agilent Technologies) and an HP-88 capillary column GC (HP 6890; Agilent Technologies). One microlitre of fatty acid methyl esters dissolved in hexane was injected in splitless mode. Gas chromatograph conditions were as follows: injector temperature, 255 °C; initial oven temperature, 75 °C, which was increased to 165 °C at 8°/min and held for 25 min, increased to 210 °C at 10 °C/min and held for 15 min, and then increased to 240 °C at 15 °C/min and held for 3 min. Total time was 64.75 min. Detector temperature was 255 °C. Nitrogen was used as the carrier gas at a flow rate of 18 mL/min, at 38 psi (1 psi = 6.894757 kPa). Fatty acids were identified by comparing retention times with those of the methylated standards (99% pure; Sigma–Aldrich) and results were expressed as a percentage of the total fatty acids identified.

Estimation of indices

To evaluate enzyme activity related to fatty acid metabolism in the mammary gland, the desaturase index (Δ^9-desaturase) was determined using the ratio between products and substrate of C14 (C14:1/(C14:0 + C14:1)), C16 (C16:1/(C16:1 + C16:0)), and C18 (C18:1c9/(C18:0 + C18:1c9)). Values were also estimated from the literature for tapir milk (Toyoda et al. 1970) for comparison.

To measure atherogenic power, the atherogenic index was estimated based on C12:0 + 4 × C14:0 + C16:0/(MUFA + PUFA), where MUFA is monounsaturated fatty acid and PUFA is polyunsaturated fatty acid, as described in Chilliard et al. (2003).

Statistical analysis

All samples were analyzed in duplicate and results are expressed as mean ± SD. Changes over time in gross composition, energy value, and fatty acid analysis were statistically evaluated with an analysis of variance (ANOVA) (MINITAB release 14.1 statistical software; Minitab Inc., State College, Pennsylvania, USA). Tukey's test (for pairwise comparisons of the means of the different lactation periods) was used to test for differences between samples. Differences were considered significant at $p < 0.05$ and $p < 0.01$.

Table 1. Gross composition of milk from lowland tapir (*Tapirus terrrestris*) over time.

	Milk		
	Colostrum ($n = 6$)	Transitional ($n = 3$)	Mature ($n = 6$)
Component (g/100 g of milk)			
Fat	12.54±7.35a*	5.75±0.78b*	2.47±0.75c*
Total proteins	19.54±4.55a*	7.37±0.97b*	6.34±1.38b*
Whey proteins	11.80±1.48a*	3.64±0.85b*	2.41±1.06b*
Carbohydrates	2.45±0.16a	3.65±0.45b	5.41±1.07c
Casein	7.34±3.75a	3.72±0.32b	3.93±0.34b
pH	6.99±0.03a	7.03±0.06a	7.02±0.10a
Gross energy (kcal/100 g of milk)	282.1±103.7a*	92.49±24.8b	80.99±2.94b*

Note: Values are expressed as mean ± SD. Different letters within a row indicate statistically significant difference over time at $p < 0.05$.

*Statistically significant difference over time within a row at $p < 0.01$.

Results

Milk composition and energy value

The gross composition of tapir milk is shown in Table 1. Fat and protein content decreased during lactation, the highest value for both being determined in the colostrum samples (fat = 17.34 g/100 g of milk; total protein = 19.5 g/100 g of milk). Fat content also showed great variation among colostrum samples ranging from 7.4 to 23.0 g/100 g of milk. Fat content reached 5.75 g/100 g of milk and total protein reached 7.37 g/100 g of milk in transitional milk samples. Mature milk had the lowest fat (2.47 g/100 g of milk) and protein (6.34 g/100 g of milk) contents. In contrast, carbohydrate content increased over time, ranging from 2.45 g/100 g of milk (colostrum) to 5.41 g/100 g of milk (mature milk). pH value was similar in all periods, with a mean value ranging from 6.99 to 7.03. The gross energetic value varied from 81.0 to 282.1 kcal/100 g of milk, with the highest value found in the colostrum samples.

Fatty acid profile

Fatty acid profile was significantly ($p < 0.05$ and $p < 0.01$) affected by lactation stage, the main differences being observed among colostrum and mature milk (Table 2). Fatty acid profile of tapir milk was characterized by high SFA content that decreased as lactation progressed: 73.2%, 68.7%, and 55.6% in colostrum, transitional milk, and mature milk, respectively. As with other perissodactyl species, capric (C10:0) and lauric (C12:0) acids were the most abundant fatty acids, with 24% and 25%, respectively, found in colostrum. Both fatty acids showed a reduction in mature milk, which was about half of that found in colostrum. Traces of caproic acid (C6:0; 0.2%–0.3%) were found in all samples. Low levels of caprylic (C8:0) and myristic (C14:0) acids were found, with a mean value over time of 2%–4.6% and 3%–5%, respectively. Palmitic (C16:0) and stearic (C18:0) acids increased from colostrum (7.3% and 3.9%, respectively) to mature milk (11.2% and 7.3%, respectively).

The high concentration of pentadecanoic (C15:0) acid is perhaps the most remarkable characteristic of tapir milk. Similar values were determined for lactation periods, with a mean value of 5.9% in colostrum and 4.5% in transitional milk and mature milk.

The overall unsaturated fatty acid (UFA) content increased over time from 26.77% to 43.73%. The mean value of MUFA was lower in early (12.9%) than in middle (14.9%) and late (19.5%, $p < 0.05$) lactation periods. Oleic acid (C18:1*n*9) represented the most abundant MUFA, with a mean value of 10.6%, 13%, and 17% in colostrum, transitional milk, and mature milk, respectively.

PUFA content was also significantly affected over time, with the lowest value observed in colostrum (13.85%) and the highest value observed in mature milk (25.2%). Among PUFAs, linoleic (C18:2*n*6) acid reached a value of 7.37% in colostrum, which is about 2.4 times higher than that found in mature milk. The mean value of linolenic acid (C18:3*n*3) was similar among different lactation periods (around 6%).

Only traces of *trans* fatty acid was detected in tapir milk as conjugated linoleic acid (CLA). Therefore, only one isomer of CLA (C18:2c9,t11) was found in tapir milk, reaching different levels in colostrum (0.08%), transitional milk (0.04%), and mature milk (0.11%).

SFA/UFA and *n*3/*n*6 ratios were used to characterize the quality of tapir milk (Table 2), determining statistical differences ($p < 0.05$ and $p < 0.01$) for both over time. Milk from early lactation presented a higher SFA/UFA ratio (2.73) than other periods (mid-lactation = 1.75; late lactation = 1.24), as well as a higher *n*3/*n*6 ratio (0.88 vs. 0.27 and 0.36, respectively).

Δ^9-Desaturase activity in mammary gland and atherogenicity index

Desaturase indices in lowland tapir (Table 3) showed an important variation over time, which is directly related to changes in fatty acid content. Lower values for C14 and C16 indices were found for colostrum (0.24 and 0.11, respectively), which was statistically different ($p < 0.05$) from transitional milk (0.58 and 0.09, respectively) and mature milk (0.14 and 0.12, respectively). Nevertheless, no significant difference on C18 index was determined among evaluated periods, showing a variation range from 0.5 to 0.7.

The atherogenicity index showed a decrease as lactation progressed, with colostrum having a significantly higher value (1.73) than mature milk (0.92), while transitional milk showed an intermediate value of 1.36.

Discussion

The general composition of milk is related to genetic, nutritional, and environmental parameters. Oftedal (1984) reported that colostrum contains higher protein and lower sugar levels than mature milk, whereas fat content in colostrum could be lower, higher, or unchanged compared with fat content in mature milk. Milk fat is perhaps the most variable component showing great diversity among animals (Palmquist 2006), because different fatty acids originated from gut or cellular metabolism or incorporated by food.

Much of the available information regarding the milk of wild animals is due to opportunistic situations where changes over lactation have not been evaluated. As mentioned, studies regarding tapir milk are scarce, but are important in improving management strategies for this species. To the best of our knowledge, few studies exist that have examined the gross composition of tapir milk over time and only one study has examined the fatty acid profile of tapir milk. Pérez et al. (2010) reported the variation in gross composition of tapir milk over time in two lactating females and Ormrod (1967) studied mature milk from one lactating lowland tapir. The fatty acid profile of colostrum and mature milk was reported from a single lactating female lowland tapir by Toyoda et al. (1970). The present study reports on samples from six

Table 2. Fatty acid profile of milk from lowland tapir (*Tapirus terrestris*) compared with milk from other species of the order Perissodactyla.

	Milk from lowland tapir			Mature milk		
Fatty acid (%)	Colostrum (n = 6)	Transitional (n = 3)	Mature (n = 6)	Horse (Park et al. 2006)	Donkey (D'Alessandro and Martemucci 2012)	Rhinoceros (Osthoff et al. 2008)
C6:0	0.22±0.08a	0.23±0.09a	0.32±0.15a	ND	6.20	ND
C8:0	4.25±1.77a	2.87±0.45a	2.30±0.74a	8.05	6.76	2.97
C10:0	24.24±6.10a*	18.07±1.74a	12.79±2.65b*	8.97	10.78	25.52
C12:0	25.42±5.88a*	15.35±1.74b	11.85±1.39c*	8.72	8.78	16.52
C14:0	3.68±1.24a	5.12±1.64a	4.63±2.35a	8.5	6.98	9.57
C14:1	1.16±0.52a*	0.10±0.01b*	0.78±0.14a*	ND	0.34	ND
C15:0	5.90±1.74a	4.50±0.96a	4.50±0.67a	ND	0.23	0.35
C16:0	7.32±2.20a	13.53±0.90b	11.18±2.06ab	23.3	19.94	15.75
C16:1	1.15±0.20a	1.26±0.26a	1.54±0.35a	3.96	2.79	1.16
C17:0	0.28±0.08a*	0.22±0.03a*	0.72±0.1b*	ND	0.22	0.46
C18:0	3.89±2.57a	8.79±4.34a	7.30±2.00a	1.55	1.82	8.86
C18:1*n*9c	10.64±1.24a	13.43±3.31ab	17.19±3.43b	13.72	16.65	8.56
C18:2*n*6c	7.28±1.55a*	16.91±3.42b	17.89±3.91b*	7.53	9.76	3.71
C18:2c9,t11 (CLA)	0.08±0.04a	0.04±0.02a	0.11±0.07a	ND	ND	ND
C18:3*n*6	0.09±0.0a	0.10±0.04a	0.22±0.0b	0.61	ND	ND
C18:3*n*3	6.42±1.57a	9.36±1.34a	6.67±1.18a	20.12	6.19	2.48
Saturated (SFA)	73.22±5.60a	68.70±2.88ab	55.61±12.06b	53.36	51.98	80.37
Unsaturated (UFA)	26.77±3.56a	31.82±6.92ab	43.73±6.29b	46.64	48.02	16.67
Monounsaturated (MUFA)	12.91±1.56a	14.69±1.66ab	19.51±3.93b	18.37	28.00	10.48
Polyunsaturated (PUFA)	13.85±2.44a	17.13±5.62ab	25.16±5.41b	28.26	20.02	6.19
PUFA *n*3	6.42±1.57a	5.87±1.42a	6.67±1.19a	20.73	7.12	2.5
PUFA *n*6	7.37±1.56a*	14.86±3.05b*	18.31±4.10b*	7.53	12.9	3.7
*n*3/*n*6 ratio	0.88±0.25a*	0.27±0.18b*	0.36±0.28b	2.75	0.59	0.66
SFA/UFA ratio	2.79±0.52a*	1.75±0.41ab	1.24±0.55b*	1.14	0.92	4.81

Note: Values are expressed as mean (±SD) percentage of total fatty acids. Different letters within a row indicate statistically significant difference at $p < 0.05$. UFA, unsaturated fatty acids (MUFA + PUFA); ND, not detected.

*Statistically significant difference within a row at $p < 0.01$.

Table 3. Indices of desaturase and atherogenicity in milk from lowland tapir (*Tapirus terrestris*) over time.

	Milk			Estimated from data in Toyoda et al. (1970)	
Index	Colostrum (n = 6)	Transition (n = 3)	Mature (n = 6)	Colostrum	Mature
Atherogenicity	1.73±0.38a	1.36±0.27ab	0.92±0.10b	4.05	2.13
Δ^9-Desaturase					
14/14+14:1	0.24±0.14ab	0.58±0.36bc	0.14±0.06a	0.01	0.02
16/16+16:1	0.11±0.06a	0.09±0.09a	0.12±0.09a	0.15	0.11
18/18:0+C18:1c9	0.74±0.13a	0.54±0.36a	0.70±0.45a	0.83	0.77

Note: Values are expressed as mean (±SD) percentage of total fatty acids. Different letters within a row indicate statistically significant differences at $p < 0.05$.

lowland tapirs and compares them with data found in the literature on tapirs and other perissodactyl animals with close phylogenetic relationship to tapirs.

The higher protein content reported during the first days of lactation is due to lactogenesis, which is intended to meet the newborn's nutritional requirements. Colostrum provides all the immunoglobulins and enzymes that prevent infection. Casein content, as well as other minor proteins, is twofold higher in colostrum than in mature milk. Changes over time of fat, protein, and carbohydrate content were similar to results reported by Pérez et al. (2010) for tapirs. Casein is the most abundant protein in ruminant milk; equid milk, however, contains less casein and more whey proteins (Uniacke-Lowe et al. 2010) as reported for tapir milk.

The higher pH value that we found in our study is similar to findings for perissodactyls reported by Zainuddin et al. (1990) and Osthoff et al. (2008) that were due to low casein content.

Colostrum samples had the highest gross energy value compared with transitional milk and mature milk samples, which is due to their high fat and protein contents. Colostrum is thus the perfect source of energy for newborns; e.g., the body mass of a tapir calf increased by 0.6 kg/week during consumption of colostrum. Fat content trended to decrease over time, which is similar to data reported in the literature for tapir (Pérez et al. 2010) and other perissodactyls (Mariani et al. 2001; Martemucci and D'Alessandro 2012).

The fatty acid profile of tapir milk was less diverse in components than that of ruminant milk but was similar to milk from other nonruminant mammals (Table 2); the fatty acid profile of tapir milk from C6 to C18:3 was similar to that of milk from horses, donkeys, and rhinoceroses (Salimei and Fantuz 2012; Pietrzaćk-Fiecko et al. 2013; Osthoff et al. 2005, 2008). Although Malacarne et al. (2002), Salimei et al. (2004), and Salimei and Fantuz (2012) detected butyric acid (C4:0) in equid milk and Toyoda et al. (1970) detected low levels (0.3%) of C4:0 in tapir milk, we did not detect this fatty acid.

The predominance of SFA in our results was similar to that reported in the literature for tapir milk (i.e., 80.4% for colostrum and 60.6% for mature milk) by Toyoda et al. (1970). Milk from perissodactyls and elephants are both characterized by high amounts of C10 and C12 (Linzell et al. 1972, Osthoff et al. 2007, 2011), which was also observed in the present study. Moreover, these high C10 and C12 values are similar to that reported by Toyoda et al. (1970). The decreasing values of C10 and C12 during lactation in lowland tapir over time in our results is only similar to data reported for horse milk by Park et al. (2006); our values are

Table 4. Comparison of the gross composition of milk among different species of the order Perissodactyla.

	Lowland tapir				
	Present study	Literature*	Horse†	Donkey‡	Rhinoceros§
Component (g/100 g of milk)					
Fat	2.47	3.4–4.1	1.21–2.6	0.53–1.4	0.74–0.99
Protein	6.34	5.7–5.9	2.14–6.4	1.59–1.72	1.62–4.17
Carbohydrate	5.41	5.3	4–7.4	6.44–6.88	5.38–8.0
Casein	3.93	4.8	1.07–1.3	0.87–1.0	0.29
Whey protein	2.41	1.1	0.83–1.2	0.68–1.0	1.33
Casein:whey ratio	1.6:1	4.3:1	1.5:1	1.5:1	0.2:1

Note: Results of the present study are mean values for mature milk.

*From Ormrod (1967) and Toyoda et al. (1970).

†From Doreau and Martuzzi (2006), Oftedal et al. (1983), Oftedal and Jenness (1988), Salimei et al. (1996), Hoffman et al. (1998), and Marconi and Panfili (1998).

‡From Oftedal and Jenness (1988), Salimei et al. (2004), Chiofalo et al. (2005), Salimei and Chiofalo (2006), and D'Alessandro and Martemucci (2012).

§From Zainuddin et al. (1990) and Osthoff et al. (2008).

different compared with values reported for milk from rhinoceroses (Klös et al. 1972, 1974) and elephants (Osthoff et al. 2005). Another remarkable fact was the extremely high C15:0 content in tapir milk in our study. Although differences in its content were reported for perissodactyl mammals and elephants (Marconi and Panfili 1998; Doreau and Martuzzi 2006; Salimei and Fantuz 2012; Osthoff et al. 2011), such a high C15:0 content has never been reported in milk from other nonruminants. However, Toyoda et al. (1970) reported high C15:1 content in colostrum and mature milk, which has also never been reported in milk from ruminants. The sum of both C15 and C15:1 content gives a similar value to our results (2.9% in mature milk). The activity of a specific thioesterase enzyme involved in fatty acid synthesis in tapirs must be carefully evaluated to explain the lipid profile that we obtained in our results.

The MUFA content in our study was similar to data reported in the literature for tapirs (Toyoda et al. 1970) and donkeys, but was lower than those reported for milk from horses (Salimei et al. 2004; Salimei and Fantuz 2012) and rhinoceroses (Osthoff et al. 2008). However, total PUFA content was 2 times higher in colostrum and 1.4 times higher in mature milk compared with the data reported by Toyoda et al. (1970). Among PUFAs, linoleic and linolenic acids are considered essential because of their important biological function (Rajamoorthi et al. 2005; Arterburn et al. 2006). They are precursors of long-chain PUFA and are considered structural components of cellular membranes. Although long-chain PUFAs were not found in tapir milk, several authors reported low levels in milk from donkeys (Salimei et al. 2004) and horses (Salimei et al. 1996). Another PUFA considered to be an important bioactive lipid because of its anticarcinogenic, antidiabetic, and immunemodulator properties (Van Nieuwenhove et al. 2012) is the conjugated linoleic acid (CLA). Ruminant milk, containing between 0.2% and 1.2% CLA, is the best source of CLA (Van Nieuwenhove et al. 2009, 2012), whereas equid milk is almost CLA-free. Values less than 0.09% was measured in horse milk (Malacarne et al. 2002), which is similar to the results of the present study. Traces of CLA, measured in tapirs for the first time, represent the only *trans* fatty acid observed in tapir milk.

Large differences among lipid content and fatty acid composition between ruminants and nonruminants were observed by Devle et al. (2012), who reported that milk from monogastric animals is characterized by high PUFA content (e.g., 19.3% and 14.2% PUFAs in milk from horses and donkeys, respectively). Our results, however, were higher than these for mature milk from tapirs.

The increasing C18:1 and C18:2 contents observed in the present study is similar to results reported by Toyoda et al. (1970); however, they were not similar to those for other related species such as rhinoceroses (Klös et al. 1972, 1974). However, studies involving wild species included only a few samples, which made it difficult to fully understand a particular animal's physiology. More studies are required to establish the fatty acid metabolism involved in the different fatty acid patterns that we observed.

The final proportion of different fatty acids reported in mature milk (approximately 50% SFA and 50% UFA) was similar to that in equid milk (Malacarne et al. 2002; Martemucci and D'Alessandro 2012); however, they were not similar in milk from rhinoceroses (83.3% SPA and 17% UFA) or African elephants (84%–97% SFA and 2.8%–16% UFA) (Osthoff 2012). The SFA/UFA ratio in early- and late-lactation periods was lower than the data reported in the literature (Toyoda et al. 1970). However, the mean SFA/UFA ratio for mature milk was slightly higher than that for donkeys (0.86−0.92) (Salimei et al 2004; Martemucci and D'Alessandro 2012), and was similar to the mean SFA/UFA ratio for mature milk from horses (1.21) (Orlandi et al. 2003).

Compared with other mammals, the $n3/n6$ ratio estimated for the late-lactation period was lower than that reported for donkeys (0.59) by Martemucci and D'Alessandro (2012). However, in milk from horses, a wide range in variation from 0.57 to 1.40 was reported by Salimei and Fantuz (2012). Because no particular fatty acid was identified in tapir milk by other authors, estimating the $n3/n6$ ratio was not even possible.

The mammary gland has substantial Δ^9-desaturase activity, which can be indirectly measured by comparing the product/substrate ratio of certain fatty acids. Therefore, the C16:1/(C16:1 + C16:0) ratio could be a good indicator in milk according to Lock et al. (2005), because C16:1 was generally abundant in milk. Moreover, the C14 desaturase index could be used because all C14 arises from de novo synthesis in the mammary gland. The complimentary C18 index was also estimated. Our results of indices of desaturase and atherogenicity in tapir milk were compared with data reported by Toyoda et al. (1970) (Table 3). Though, our results are similar for C18 and C16 but significantly higher for C14 indices. Compared with milk from donkeys, milk from tapirs contained higher C14 (0.14 vs. 0.04, respectively), similar C16 (0.12 vs. 0.14, respectively), and lower C18 desaturase index (0.70 vs. 0.93, respectively) according to Martemucci and D'Alessandro (2012). The large variability in desaturation activity could explain the high variation of some MUFAs as lactation progressed.

The estimated value of the atherogenic index for milk from tapirs cited in literature was 4.05 for colostrum and 2.13 for mature milk, which are both two times greater than our results. However, we obtained similar values to that reported by Martemucci and D'Alessandro (2012) for milk from donkeys (1.16). The atherogenic index followed the SFA pattern, whereby SFAs decreased while UFAs increased over time, and reached a mean value that was two times greater for colostrum than for mature milk.

The comparison of gross composition of milk with previous studies on tapirs and other perissodactyl species is shown in Table 4. Only values for mature milk were analyzed. Note that perissodactyl species exhibit a large range in gross composition of mature milk. The fat content determined in the present study was lower than those previously reported for tapir (3.4–4.1 g/100 g of milk), but similar to fat content of milk from horses and higher than fat content of milk from donkeys and rhinoceroses. We also obtained slightly greater amounts of total protein compared with data previously reported for tapir milk. However, our result was similar to protein content of milk from horses, but higher than that from donkeys and rhinoceroses. Carbohydrate content seems to be the less variable compound among species, with our value being similar to that reported by other studies for milk from tapirs, horses, and rhinoceroses. Casein content of milk in our study was less than that reported for tapirs by other studies, but was still the highest value among other perissodactyls. Whey protein content of milk in our study was also higher than that previously reported for tapirs and other perissodactyl species. However, the final casein:whey ratio from tapir milk (1.6:1) in our study was similar to that from horses and donkeys (1.5:1), but higher than that from rhinoceroses (0.29:1). However, our casein:whey ratio was less than that reported for tapirs by Toyoda et al. (1970).

The long interval between pregnancies (close to 2 years) and the long gestation period of tapirs make it difficult to obtain more milk samples to examine. However, very consistent results were obtained in our study, which was the first time that considerable amount of samples from tapirs was included in a study. It is remarkable that we evaluated the milk composition through lactation of six tapirs from different regions of Argentina that were subjected to different management strategies. However, no differences were found between captive and semicaptive animals among gross composition of milk and fatty acid composition (data not shown).

The information about milk composition from wild animals is important to our understanding of the nutritional demands of newborns, especially for wild species in danger of extinction that have calves in captive or semicaptive conditions. Tapirs have not received a lot of attention compared with other wild, closely related perissodactyls. Thus, our results contribute to the knowledge behind tapir physiology, as well as contributing to our understanding of milk from other members of the order Perissodactyla. Our study adds to the knowledge of tapir fatty acid metabolism and provides information to manipulate feeding strategies for the management of semicaptive or captive conditions. Our study is only the beginning and further studies are underway in our laboratory that will examine the activities of casein, minerals, and enzymes in tapir milk.

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