TAPIR MILK DIFFERS IN NUTRIENT COMPOSITION FROM EQUID AND RHINOCEROS MILK

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Introduction

Perissodactyla is a mammalian order known as odd-toed ungulates. The order consists of Equidae, Rhinocerotidae, and Tapiridae. The data presented is part of a larger paper exploring the milks of the Perissodactyla class, comparing their nutrients to determine if there is a phylogenetic constraint on milk composition.

Milk is a fundamental evolutionary adaption by mammals and is the main source of nutrients and hydration for mammalian offspring. Milk is the starting blocks for any mammal's growth and development. Milk composition can vary widely between mammal species, but the nutritional composition of milk often appears to follow phylogeny, with milks of closely related species being similar to each other. However, other factors such as litter size, lactation strategy, neonatal growth rate, and adult diet are also associated with milk nutrient composition.

Present data indicates that there is a phylogeny trend of high sugar milk in Perissodactyla based on published data from equids (Oftedal, 1983) and rhinos (Osthoff, 2021; Table 1). The Smithsonian milk repository has milk samples from a total of 5 equid species, 3 rhino species with a total of 16 females, with various longitudinal samples that are a part of the broader project. Equids and rhinos in general have very similar behaviors. They are megaherbivores that graze in open plains.

Tapirs in comparison, are smaller herbivores with unique trunk-like snouts that roam in tropical forests. Tapirs are both grazers and browsers who consume fruits as well as aquatic plants. Tapirs spend a large amount of time in water, similar to hippo or pig behaviors. Tapir morphology has changed little since their ancestors 40 million years ago. A published report of tapir milk suggests that their milk differs in composition from equid and rhino milk (Van Nieuwenhove, 2014). This report examines this hypothesis using more extensive longitudinal milk samples from Baird's tapirs.

Materials and Methods

Sample Collection

Milk samples were selected from the Smithsonian Milk Repository located at the National Zoological Park's Nutrition Lab. The milk samples were screened for accurate metadata and were selected based on the species, female, and the collection date postpartum. Samples came from captive animals. Overall, 15 samples were selected from 2 different Baird's tapir females. All samples had been thawed at least 2 times before being stored in a -20°F freezer.

Nutritional Analysis

A full milk nutrition composition analysis will be completed for dry matter, fat, crude protein (CP), total sugar, gross energy (GE), ash, calcium, and phosphorus at the Smithsonian National Zoological Park Nutrition Laboratory (Washington, DC) following the lab's standard protocols

(Oftedal & Iverson 1995; Hood, 2009). Crude Protein will be calculated by drying milk and running samples through a CHN analyzer (Model 2400; Perkin Elmer, Waltham, MA). CP is calculated by multiplying total nitrogen by the conversion factor 6.38 (Jones, 1931). Fat content will be measured with a microfat Rose-Gottlieb procedure that was modified (Hood *et al*., 2009). Total sugar will be measured through a phenol-sulphuric acid colorimetric procedure (Dubois *et al*., 1956; Marier & Boulet, 1959) using lactose monohydrate standards and read at 490 nm on a microplate reader (Model ELX808; BioTek, Winooski, VT). Ash will be determined by placing dried milk in a muffle furnace at 550°C for 8 h. Phosphorus will be determined by the AOAC-Modified Gomorri colorimetric method and read with a microplate reader and accompanying software (MRX TC Revelation) at 450 nm (Gomorri 1942; AOAC 1990). Calcium will be measured using atomic absorption spectrophotometry (Model 800 Perkin Elmer Analyst Flame Furnace Atomic Absorption Spectrophotometer; Perkin Elmer Co., Waltham, Massachusetts) at 422.7 nm using a nitrous oxide flame (AOAC, 1990). Dry matter was measured and calculated when drying the milks while subsampling for CP analysis and Ash. The GE formula used (GE = (9.11 kcal/g * % fat + 5.86 kcal/g * % crude protein + 3.95 kcal/g * % sugar)/100. To convert to kJ/g, multiply by 4.184 kJ/kcal) has been validated against GE values measured by bomb calorimetry for milk from several species, including rhesus macaques (Hinde, 2009), bongo (Petzinger, 2014), and African elephant (Himschoot *et al*., 2021). This equation has the potential to slightly overestimate GE because it fails to correct for nonprotein nitrogen (Oftedal, 1984).

Statistical Analysis

Averages of the milk samples nutritional composition percentage and mg/kcal GE was calculated, and the longitudinal samples were analyzed with a linear regression.

Results

Results are still pending and may vary as we continue our analysis. The DM% ranges between 10.05% and 28.89% while the general average is 15.46%DM. Tapir milk sugar percentage according to past literature and our recent findings is averaging around 4.73% sugar. There also appears to be a slight trend of increasing % sugar over time, consistent with declining DM (increasing water content; Table 2).

Discussion

Tapir milk composition appears to differ from milks of the other two Perissodactyla families. The tapir's have a significantly lower percent sugar in their milk compared to rhinos and horses, whose milk ranges more around 7% sugar. In addition, tapir milk appears to have on average a higher %DM. We predict that fat, protein, and GE of tapir milk will all be higher than the values of the milks from equids and rhinos. This indicates that, although there are some phylogenetic similarities among the order, tapir milk has evolved to differ in nutritional composition from the other two families. The difference is likely due to the difference of environmental pressures and diet of feeding in forested areas comparatively to open plains.

More conclusions will be made or altered as more data is revealed. It will be interesting to discover if the sugar % is lower in tapirs, what other nutrient is higher than the rest of the Perissodactyla class, and if that nutrient is consistent to the conclusions made of its milk reflecting the tapir's difference in diet and environment.

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Table 1. A sample of literature values for equid and rhinoceros milk nutrient composition.

Species	***n***	**DM, %**	**Fat, %**	**Protein, %**	**Sugar, %**	**Ash, %**
Horse[1]	5	10.50	1.29	1.93	6.91	N/A
Rhinoceros[2]	15	9.37	1.76	0.93	7.93	0.40

[1]Oftedal et al. (1983)
[2]Osthoff et al. (2021)

Table 2. Baird's tapir milk samples used in this longitudinal study with dry matter percentage and sugar percentage.

Female	**Days Postpartum**	**DM, %**	**Sugar, %**
A	3	28.89	
	6	19.10	
	9	16.73	3.76
	13	16.38	
	19	13.31	
	25	16.07	4.15
	31	12.29	
	44	16.33	4.80
	69	10.05	
	82	13.28	4.93
	96	11.32	
	103	11.79	5.05
B	11	14.04	
	17		
	24		
	60	11.77	