Investigation into the Nutritional Content and Microbiological Property of Abyssinian Donkey's Milk

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Abstract

A study was carried out on donkey milk in Ada'a District of central Ethiopia to investigate the nutritional and microbiological properties of Abyssinian donkey's milk. The study utilized primary data that were collected from 24 jennies in the study area. The physicochemical composition such as fat, total protein, lactose, minerals, vitamin C, pH, density, total solid and freezing points; and microbiological characteristics were analyzed. Results indicated that the Abyssinian donkey milk has close similarity with milk of other donkeys previously investigated elsewhere for most of the compositions. More importantly, the present findings confirmed that Abyssinian donkey milk has a very close similarity with human breast milk in its protein, lactose, vitamin C, pH, density, and zinc contents whereas, the concentrations of calcium, iron and magnesium were higher than those of human breast milk. Other unique properties of Abyssinian donkey milk were lack of fermentation and lower microbial load in contrast to cow's milk. In conclusion, the findings of this preliminary study showed the existence of similarity between Abyssinian donkey's milk and human breast milk in their physicochemical composition and thus could suggest that the Abyssinian donkey's milk can serve as an alternative supplement for human breast milk despite differences in some aspects of the microbiological and sensory properties. However, Abyssinian donkey's milk should be tested for its safety to human being before it is recommended for human consumption.

Key words: donkey milk, Ethiopia, human breast milk, physicochemical property

Introduction

Milk production from dairy cows in Ethiopia is among the lowest in the world; 1.54 liters per cow per day (Tesfaye Lemma Tefera *et al*, 2010). Contributions from the different livestock are in the order of 81.2%, 6.3%, 7.9% 4.6% from cattle, camels, goats and ewes, respectively (CSA, 2010). The per capita milk consumption (16 kg/year) has not changed much to date and is much lower than reports for Africa (27 kg/year) and developed world (100kg/year), respectively (Saxena *et al*., 1997). There is a clear gap between the demand and supply in the face an additional per annum demand of 6 million tons of milk (Saxena *et al*., 1997). Low palatability, protein related allergies, unfavorable immune responses, hyperlipidemia, and gut intolerances continue to limit cow's milk preference for infants, children, newly suckling mothers, and the elderly. As a result, mare and donkey milk has been produced and commercialized in some parts of the world (Nikkhah, 2012; Salimei and Fantuz, 2012).

In Ethiopia, one of the leading causes of infant mortality is malnutrition. The ever rising scarcity and high cost of milk has made it impossible to meet the demand. One-fourth of women in Ethiopia are malnourished, and approximately 50% of children under five years of age are moderately or severely stunted partly because of failure of breast feeding during the first months of their infant life (CSA, 2010). Furthermore, one third of babies do not receive breast feeding within one hour of birth (Meftuh *et al*., 1990). Reports also showed that malnutrition, even in its milder forms, accounts directly or indirectly for 53% of under-five deaths in Ethiopia (Burns, 2001). Moreover, due to the highly perishable nature of milk and mishandling, losses of up to 20–35% have been reported (Zegeye Yigezu, 2003). Donkey milk has long been praised for its unique nutritional quality and its similarity to human milk. Consequently, donkey milk has been recently considered as a substitute for cow's milk protein (Tesse *et al.,* 2009). Ethiopia with its 6.5 million donkeys has a good chance for enhancing donkey milk production. Donkey milk has a unique protein, fat, carbohydrate and ash content, and low microbial count (Salimei *et al*., 2004; Chiavari *et al*., 2005). Donkey's milk has been used for feeding infants in different regions of the world (Tesse *et al*., 2009; Salimei and Fantuz, 2012) for treatment of diseases (Vincenzetti *et al*., 2008) and for cosmetics (Guo, *et al*., 2007; Vincenzetti *et al*., 2008).

The physicochemical compositions of milk from different animals are known to vary based on feeding condition, breed and locality. In this regard, no data is available on the composition and microbiological properties of donkey milk in Ethiopia. Therefore, the objective of this study was to characterize the physicochemical composition and microbiological properties of Abyssinian donkey milk.

Materials and Methods

Study area and study animals

This study was conducted in Ade'a District of Oromia Regional State located about 45 km southeast of Addis Ababa at $8^0 7^0$N latitude and 39^0E longitude, at an altitude of 1990 meters above sea level. It receives annual rainfall of 866 mm with the long rainy season extending from June to September accounting for 84% of the precipitation. The mean annual maximum and minimum temperature ranges are 26^0C and 14^0C, respectively. According to the local agricultural office, there are about 9845 donkeys in the District and they are mainly engaged in agricultural product transport operation.

Study Design

Approximately 500 ml milk samples were aseptically collected directly into acid rinsed, sterile container from 24 healthy jennies. The milk samples were immediately transported using cool box at +8°C within one hour of collection. Physical analyses and pH were analyzed soon after collection upon arrival at the laboratory. Further, milk samples from other 6 jennies was added for microbiological analysis. The microbiological analysis was carried out at Ethiopian Health and Nutrition Research Institute (EHNRI) at the Department of Drug Research and Institute of Biodiversity while all the remaining analysis was carried out at the Ethiopian Standard and Quality Authority Laboratory.

Total solid was determined using IDF/ISO-AOAC (International Dairy Federation/International Organization for Standardization-Association of Official Analytical Chemists) method. Ca, Mg, Fe, and Zn were determined using Standard AOAC method (Varian, Spectra 220). Vitamin C and Phosphors were determined using Spectrophotometer (UV-v3, Thermo Electrocorporation). Fat content was determined using Gerber method while protein content was determined using the Kjeldahl methods.

The samples for microbiological analysis were collected from Hiddi, GaraBoru and Babogaya areas within Ade'a District. Fermentation was then initiated with the leftover samples and allowed to proceed for up to 18 days at room temperature. For the microbiological analysis, plate count agar (PCA), potato dextrose agar (PDA), M17 (M17- Agar), Mann Rogosa Sharpe agar (MRSA), and MacConkey agar were used to cultivate and isolate total aerobic bacteria (TAB), fungi (yeast and mould), gram positive and gram negative microbes, respectively. Incubation was carried out at 30 °C for M17 and MRS plates; at 37 °C for PCA and MacConkey plates; and at 25 °C for PDA plates. Plates were examined for colony and colony forming units per milliliter (cfu/ml) of test samples were calculated. Cultural characteristics, morphology and results of biochemical tests (Catalase and Oxidase test) were recorded.

Statistical Analysis

Data were analyzed by SPSS (version 15/2007, USA). This test combined ANOVA with comparison of differences between means of the location. One-way ANOVA was conducted to test location differences. Correlation analysis was also performed to observe the relationship of milk parameters under investigation. It was assumed to show presence of a good correlation when $r \geq 0.26$ and the level of significance was kept at $P< 0.05$.

Results

Physical analysis

Summary of the physicochemical analysis are given in Table 1. The (Mean ± SD) Total solid, pH, Density, Acidity, and Freezing point of the milk sample were 8.80 ±0.98%, 7.20±0.12, 1.026 ±0.006g/cm^3, 0.14 ±0.15% and -0.45±0.066^{0}C, respectively. PH level did not vary much (CV= 0.02) among individual donkeys while acidity was the most varying (CV=1.07) parameter.

Proximate analysis

Summary of the chemical composition analysis is given in Table 2. Lactose level was found to be the least varying milk component while vitamin C and fat were the most varying milk components among individual donkeys.

Table 1: Chemical composition of donkey milk samples collected from study sites

Item	N	Mean (±SD)	CV*	Minimum	Maximum
Lactose [%]	24	5.39± 0.45	0.07	5.39	6.29
Fat [%]	24	0.37 ±0.11	0.31	0.23	0.67
Protein [%]	24	1.98±0.34	0.17	1.43	2.89
Vitamin C	24	3.39 ± 1.57	0.46	1.25	6.23

CV= coefficient of variation*

Mineral Analysis

The results of the standard AOAC analysis the mean (±SD) levels of Mg, and Zn to be 58.7 ± 22.4 mg/ml and 5.56 ±3.38 mg/ml, respectively. Ca (737 ± 309 mg/ml) was the highest and most abundant mineral while phosphorus (314 ± 151 mg/ml) was the least abundant mineral. There was a high variability in Fe (21.3 ± 28.4 mg/ml; range=9. 48- 94.7) content among individual donkeys.

Iron and zinc contents of the milk were significantly (P=0.004) differed among the samples collected from the three sites. Samples from Garaboru had relatively lower pH (7.1), higher density (1.04g/cm^3) and higher concentration of iron (38.6 mg/ml), whereas samples from Hidi had higher pH (7.3), and lowest density (1.01g/cm^3). Concentrations of both iron (9.5 mg/ml) and zinc (3.8 mg/ml) were lowest in samples collected from Babogaya. The remaining milk components were not significantly different among the study sites.

Microbiological analysis

The overall mean TAC, fungi, Gram negative (GN), and Gram positive (GP) bacteria for the three sampling site was 4.75log cfu/ml, 1.94cfu/ml, 4.5 cfu/ml, and 2.85 cfu/ml, respectively. Results of the microbiological analysis for the different study sites are summarized in Table 2. At room temperature, TAC, GP bacteria and yeast increased progressively during the 18 days storage while GN bacteria and mold decreased under the same conditions. After the morphological assessment; and catalase and oxidase tests a total of 20 bacterial isolates (Coccii, diplo Coccii, Very short rod, Short rod and Rod) were identified as lactic acid bacteria which were further identified at species level by OmniLogPuls Identification.

Table 2: Mean value of microbial count for TAB, GN and GP bacteria, yeast and mold in donkey milk samples collected from the three study sites

Location	Time of incubation	Expected microbial group				
		TAB* [log cfu/ ml]	GN [log cfu/ ml]	GP [log cfu/ ml]	Yeast [log cfu/ ml]	Mold [log cfu/ ml]
Garaboru	0 hr	5.66	5.40	2.32	1.72	2.45
	18 days	6.32	4.81	3.38	4.69	1.40
Babogaya	0 hr	4.62	5.01	3.08	1.18	1.25
	18 days	6.00	4.48	6.07	1.00	1.14
Hiddi	0 hr	3.99	3.11	3.15	2.72	2.30
	18 days	6.20	2.77	6.74	1.48	1.00
Overall	0 hr	4.75	4.50	2.85	1.87	2.00
	18 days	6.17	4.02	4.75	2.39	1.18

TAB= Total aerobic bacteria; GN= Gram negative; GP= Gram positive*

Only three isolates (DM-29 and DM-120a both *Clavibacter agropyri* and DM-150 which is *Leifsonia aquatica*) showed successful growth on the OmniLogPlus ID System. However, the remaining organisms pre-isolated as lactic acid did not grow on OmniLogPlus ID System agar. Unlike previous assumptions and as it occur in the cow's milk, donkey milk doesn't ferment under natural condition, but an insignificant drop in pH can be seen due to the action of microorganism. Incubation for 18 days resulted in a relatively unchanged pH values rather than fermentation.

Discussion and Conclusion

The physicochemical and microbiological characteristics of Abyssinian donkey's milk were investigated in this study. Generally, Abyssinian donkey milk has a high freezing point, very low fat content, a high calcium, iron and vitamin C content. Moreover, it is also characterized by a very low microbial content. The milk was relatively more acidic than human milk (Salimei *et al*., 2004) but closely similar (Acidity = 0.14 to 0.16%) to cow's milk (O'Connor, 1995). The pH was also similar to previous reports for donkeys elsewhere (Guo *et al*., 2007) but distinctively higher than the 6.89 ± 0.08 and 6.3 ±6.8 reported for human and cow's milk, respectively (Van den Berg 1988; Guo *et al*., 2007). On the other hand, the density was within the range of reports for other donkeys (1.03 ± 0.02) and for human milk (1.027 ± 0.011) (Guo *et al*., 2007).

The dry matter content, though similar with donkeys elsewhere (Salimei and Fantuz, 2012), was much lower than that of human milk (Chiavari *et al*., 2005). The freezing point was also relatively higher compared with that of cow's milk (Samia *et al*., 2009). The findings of the present study generally agrees with previous suggestion that donkey's milk may be considered a valid alternative for infant nutrition, to powdered milks, soybean milk or other formulas, since its composition in lipids and proteins is very similar to human milk (Salimei *et al*., 2004, Vincenzetti, *et al*., 2008). Conversely, the fat content was lower compared to the 0.86% reported by Chiofalo and Salimei, (2001) and even much lower than the 2.1± 0.15% reported for human milk (Salimei *et al*., 2004). Both lactose (the least varying milk component) and Vitamin C (the most varying milk component) were closely similar to concentrations reported both for human and donkey milk elsewhere (Luthfor *et al*., 2004; Guo *et al*., 2007).

The concentration of Ca was nearly twice as high as the 334.61mg/kg for donkey and 340.0 mg/kg for human milk later reported by (Salimei *et al*., 2003). It is also relatively higher than that of cow's (680 ± 79.8 mg/k) and goat's milk (646 ±76.6 mg/kg) (Salimei *et al*., 2004). The concentration of Mg was much lower than that of goat's milk (139 mg/kg) and cow's milk (205 mg/kg) (Mohammad *et al*., 2008) while it was found to be much higher than that of human milk (Dorea, 2000; Salimei *et al*., 2004).

The concentration of Fe was several folds higher than those reported both for human (0.4 ± 0.01mg/kg) and donkeys elsewhere (1.15±0.51 mg/kg) (Salimei *et al*., 2004). On the other hand, P was lower than other donkeys (487 mg/kg) but much higher than human milk (160± 0.85 mg/kg). Variations in iron and zinc level, milk pH and density among samples from different study sites might show the presence of differences in feed types which is mainly derived from the natural pasture. As indicated previously, raw donkey milk contains a very low microbial content compared to that of bovine milk. This could be due to the presence of antimicrobial activity of donkey milk because of its high lysozyme content (Mao *et al*., 2009).

In conclusion, the present study demonstrated a general resemblance, with some differences, in the gross composition and physicochemical characteristic between milk from Abyssinian donkey and donkeys elsewhere. Further, similarities have also been observed with human milk. Except for Zn and Fe, which were much higher for Abyssinian donkey milk there were only minor difference in other minerals from donkeys studied elsewhere. Organism identified as lactic acid bacteria were not capable of fermenting donkey's milk. Further, the low microbial counts and absence of fermentation of the raw milk might indicate the potential use of donkey milk as a safe natural food for infants. As demonstrated in this study, the great resemblance between Abyssinian donkey milk and human milk should allow further investigation for use of donkey milk as human milk replacer. But, before buying this recommendation further objective researches are needed to establish the sensory and detailed antimicrobial properties of donkey milk.

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