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Composition, Physiochemical Properties, Nitrogen Fraction Distribution, and Amino Acid Profile of Donkey Milk

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ABSTRACT

This study investigated the changes in chemical composition, nitrogen fraction distribution, and AA profile of milk samples obtained during lactation from the Jiangyue breed of donkey in Northwest China. Results showed that donkey milk contained 9.53% total solids, 1.57% protein, 1.16% fat, 6.33% lactose, and 0.4% ash on average, which is more similar to mare and human milk than to the milk of other mammals. Throughout the lactation investigated, pH and density were constant, protein and ash content showed an apparent negative trend (an increase in lactose content during 120 d postpartum, followed by a decrease), fat content exhibited wide variability, and variations in the content and percentage of whey protein, casein, and AA were small. The casein to whey protein ratio of 52:37 was between the lower value of human milk and the higher value of cow milk. Sodium dodecyl sulfate-PAGE results demonstrated that donkey milk is rich in β-lactoglobulin and lysozyme. The percentages of 8 essential AA in protein of donkey milk were 38.2%, higher than those of mare and cow milk; donkey milk also had higher levels of serine (6.2%), glutamic acid (22.8%), arginine (4.6%), and valine (6.5%) and a lower level of cystine (0.4%).

Key words: donkey milk, composition, nitrogen fraction, amino acid

INTRODUCTION

Research interest and capital investment in donkey milk have increased in Europe because its composition is similar to that of human milk (Carroccio et al., 2000). However, information on the composition of donkey milk is extremely limited, whereas the milk of mares (which are closely related to the donkey) has been researched extensively.

Available data (Table 1) suggest that donkey milk shares much similarity with mare milk in being low in total solids (8 to 10%) and protein (1.5 to 1.8%) and being high in lactose (6 to 7%). The level of fat in donkey milk ranges from 0.28% (Chiavari et al., 2005) to 1.82% (Oftedal and Jenness, 1988). Its protein percentage has been characterized as having low CN (47.3%) and whey protein contents (36.9%), where β-LG accounted for just 29.85% (Salimei et al., 2004). In addition, donkey milk has been suggested to contain high concentrations of peptide-bound AA, particularly essential AA (Taha and Kielwein, 1990). Compared with the milk of Friesian cows, buffaloes, goats, ewes, camels, and mares, donkey milk had the highest levels of Val and Lys (Abd-EI-Salam et al., 1992). Furthermore, its hormonal peptides, which stimulate the functional recovery and development of the intestine, could provide growth and protective factors (Carroccio et al., 2000).

All the above facts imply that donkey milk can be used as an alternative for infants who show an intolerance to cow milk. A recent clinical study confirmed that feeding donkey milk is a safe and valid treatment for most complicated cases of multiple food intolerance (Carroccio et al., 2000). Mare and donkey milk may be used in selected cases of cow milk allergy after appropriate modification to make them suitable for human infants (Muraro et al., 2002). In addition to its use for infants, some researchers have reported that donkey milk has an effect on the osteogenesis process, as well as in arteriosclerosis therapy, for the rehabilitation of patients with coronary heart disease or premature senescence, and in hypocholesterolemic diets (Chiofalo et al., 2001).

The advantages of donkey milk have attracted considerable interest in its use for human consumption. Today, however, donkey breeding is so disperse that the milk yield is very low. With almost 8 million head of donkey in 2004, China has the largest donkey stock worldwide, followed by Pakistan and Ethiopia (FAO, 2005). Donkeys are primarily raised in Northwest China in the Xinjiang and Shanxi provinces. In the last several years, the Xinjiang province has strived to

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Table 1. Composition, physical properties, content (g/100 g), and percentage distribution of nitrogen fractions of donkey,[1] mare,[2] human, and cow milk

Item	Donkey	Mare	Human	Cow
pH	7.0–7.2	7.18	7.0–7.5	6.6–6.8
Protein, g/100 g	1.5–1.8	1.5–2.8	0.9–1.7	3.1–3.8
Fat, g/100 g	0.3–1.8	0.5–2.0	3.5–4.0	3.5–3.9
Lactose, g/100 g	5.8–7.4	5.8–7.0	6.3–7.0	4.4–4.9
Ash, g/100 g	0.3–0.5	0.3–0.5	0.2–0.3	0.7–0.8
TS, g/100 g	8.8–11.7	9.3–11.6	11.7–12.9	12.5–13.0
CN, g/100 g	0.64–1.03	0.94–1.2	0.32–0.42	2.46–2.80
Whey protein, g/100 g	0.49–0.80	0.74–0.91	0.68–0.83	0.55–0.70
NPN, g/100 g	0.18–0.41	0.17–0.35	0.26–0.32	0.1–0.19
CN, %	47.28	50	26.06	77.23
Whey protein, %	36.96	38.79	53.52	17.54
NPN, %	15.76	11.21	20.42	5.23

[1]Anantakrishnan et al. (1941); Oftedal et al. (1988); Salimei et al. (2004); and Chiavari et al. (2004).
[2]Pagliarini et al. (1993); Solaroli et al. (1993); and Malacarne et al. (2002).

enhance donkey milk production by establishing many donkey-breeding centers, leading to a great improvement in the yield of donkey milk. Annual milk production has reached up to 40,000 metric tons, ranking first in China (Yang et al., 2006), and has helped make donkey milk processing possible.

Considering its unique nutrient profile and economic potential, donkey milk could be exploited to fulfill the nutritional requirements of certain people and to increase the income of donkey farmers as well. However, little is known about domestic donkey milk (e.g., of the Jiangyue breed) from the Xinjiang province. Therefore, the objective of this study was to investigate the physicochemical parameters of donkey milk from the Jiangyue breed (TS, protein, fat, lactose, ash, AA, and pH) and to determine variations in these parameters throughout lactation.

MATERIALS AND METHODS

Animals

Seventy donkeys, 4 to 14 yr of age (120 to 300 kg in weight; parities 1 to 6), selected from one herd containing 712 Jiangyue donkeys located in Kashi, southwest Xinjiang, were used in this study. The donkeys were divided into 7 groups based on the number of days after parturition (15 ± 2, 30 ± 2, 60 ± 2, 105 ± 2, 120 ± 2, 150 ± 2, and 180 ± 2 d), with 10 animals in each group. A preliminary examination and serological evaluation were conducted to ensure that the experimental animals were in a healthy condition. The average BCS of each group, as measured on a scale of 1 to 9 (Pearson and Ouassat, 2000), was 4.8 ± 0.79, 4.7 ± 0.48, 5.1 ± 0.88, 4.8 ± 0.79, 4.7 ± 0.82, 4.9 ± 0.88, and 4.7 ± 0.82, respectively. The breeding conditions were hygenic and dry. All donkeys in the study were fed the same diet, which consisted of 1.04 kg of bran, 0.88 kg of cornmeal, 0.099 kg of oil cake, 0.98 kg of Qing Chu (a fermented and stored corn straw), 1.32 kg of clover, and 0.99 kg of wheat straw daily (average: DM 78.9%; CP 13%; crude fat 2.24%; crude fiber 18.97%; digestible energy 16.28 MJ/kg, as fed).

Sampling

All the donkeys were milked manually 4 times/d (at 0600, 1200, 1800, and 2400 h). Milk samples were frozen immediately after collection at each milking and stored at –20°C until analysis. The samples taken from the same donkey were thawed and pooled before analysis.

Analyses of TS, Fat, Ash, and Lactose

Physicochemical parameters were measured as follows: pH by a digital pH meter (pH 211 microprocessor pH meter, Hanna Instruments, Padova, Italy), and density at 15°C by means of a Quevenne lactometer. Total solids were determined gravimetrically after the milk was dried in a forced-convection oven at 105°C until a constant weight was achieved (Zhang et al., 2005). Fat percentage was measured using the Röse-Gottlieb method (AOAC, 1980). Lactose was determined according to the method of Lane and Eynor (1923). Ash content was determined after incineration in a muffle furnace at 530°C (Summer et al., 2004).

The biological value of milk protein was calculated by the method of Morup and Olesen (1976) on the basis of AA composition. Gross energy (cal/kg) was calculated with the coefficients reported by Salimei et al. (2004), namely, 9.11 for fat, 5.86 for protein, and 3.95 for lactose.

Determination of Nitrogen Fractions

Nitrogen content was determined by the Kjeldahl method. The concentrations of NPN, total nitrogen (**TN**), whey protein nitrogen (**WPN**), and CN nitrogen (**CNN**) were analyzed according to the procedure described by Guo et al. (2001). A nitrogen conversion factor of 6.38 was used to calculate protein contents of milk samples and various fractions.

SDS-PAGE Analysis

For analysis of the protein profiles of each milk sample, SDS-PAGE was used under reducing conditions according to the method of Laemmli (1970), using a Mini-Protean 3 gel electrophoresis apparatus (Bio-Rad, Hercules, CA) with a 4% acrylamide stacking gel and a 16% separating gel. A mark protein ladder (Tiangen Biotech Corporation, Beijing, China), consisting of proteins ranging in molecular weight from 14.4 to 94 kDa, was used as a molecular weight standard. A human milk sample (at d 60 postpartum) was obtained from a healthy donor. Current was then applied at a constant voltage of 80 V until samples had entered the separating gel, and voltage was then increased to 120 V until completion. Gels were stained with 0.1% Coomassie Brilliant Blue R-250 in 10% acetic acid and 40% methanol, and destained overnight in 10% acetic acid and 10% methanol with 2 buffer changes.

Densitometry

Quantitative analyses of electrophoretic separations of milk proteins were performed using the Gel-Pro Analyzer 3.1 software from Media Cybernetics (Silver Spring, MD). Images of wet gels were acquired and converted from color to gray images, and contrast was optimized. Gel image analyses (recognition of lanes and bands, calculation of the molecular weight and amount of each band) were performed automatically by the software.

Analyses of AA Composition

Thawed milk samples were thoroughly mixed and hydrolyzed using 6 *M* HCl in sealed glass ampullae for 22 h at 110°C. The hydrolyzate was centrifuged and the supernatant was used for AA analysis after filtering through a 0.22-μm syringe filter. The analysis of AA was achieved by analytical reversed-phase HPLC. A C_{18} column (WAT052885, Waters, Milford, MA) was used for AA analysis, and a 2-eluent system was used for AA separation. AccQ-Tag eluent A (Waters WAT052890) was prepared with 140 m*M* sodium acetate and 17 m*M* triethylamine titrated to pH 4.95 with phosphoric acid, and eluent B was made with 0.6 g/mL of acetonitrile (HPLC grade, Fisher A998-4, Fisher Scientific, Fair Lawn, NJ) in water. The flow rate was set at 1.0 mL/min. The detector response was monitored at 248 nm.

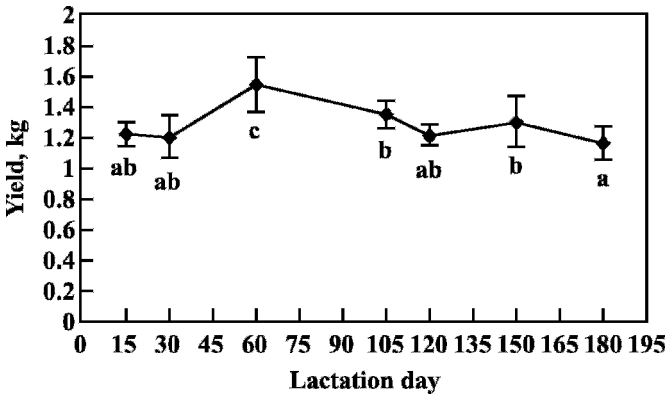

Figure 1. Milk yield per day during the 180-d lactation period (mean ± SD). [a–c]Means without a common letter are different ($P < 0.05$).

Statistical Analysis

Data were analyzed by ANOVA using SPSS software, version 12.0 (SPSS Inc., Chicago, IL). The differences between the means of the treatments were compared at a significance level of $P < 0.05$.

To assess the linear relationship between 2 parameters, simple linear correlations (Pearson's *r*) between parameters were calculated by a bivariate correlation analysis using SPSS software, version 12.0.

RESULTS AND DISCUSSION

Milk Yield

The variation in daily milk yield was moderate during the lactation period (Figure. 1). The higher milk yield observed at d 60 postpartum (**PP**) may support the hypothesis of adaptation to the milking rhythm and activity patterns of the foal. The observed trend of milk yield variation was not in agreement with that reported by Salimei et al. (2004), which was characterized by 2 transitions (at d 45 and 90 PP). In addition, the average milk yield per day calculated over the entire lactation period was 1.28 ± 0.17 kg (Figure 1), much lower than the 1.82 kg reported by Salimei et al. (2004). The difference may be attributed to the type of forage and the donkey breed.

Gross Composition of Milk

Changes in the physicochemical parameters and composition (protein, fat, lactose, and ash) of the donkey

Table 2. Physicochemical parameters and composition of donkey milk during the 180-d lactation period[1]

Parameter	Lactation stage, d							P
	15	30	60	105	120	150	180	
Yield, kg/d	1.22 ± 0.08[ab]	1.20 ± 0.14[ab]	1.54 ± 0.18[c]	1.35 ± 0.09[b]	1.21 ± 0.07[ab]	1.30 ± 0.17[b]	1.16 ± 0.11[a]	*
pH	7.21 ± 0.15	7.22 ± 0.13	7.16 ± 0.09	7.14 ± 0.08	7.16 ± 0.06	7.15 ± 0.09	7.20 ± 0.07	NS
Protein, g/100 g	1.85 ± 0.20[c]	1.72 ± 0.15[c]	1.52 ± 0.08[ab]	1.49 ± 0.14[ab]	1.37 ± 0.15[a]	1.49 ± 0.13[ab]	1.53 ± 0.11[b]	*
Fat, g/100 g	0.50 ± 0.15[a]	0.80 ± 0.22[ab]	1.32 ± 0.25[cd]	1.40 ± 0.35[de]	0.95 ± 0.28[bc]	1.43 ± 0.27[de]	1.70 ± 0.32[e]	*
Lactose, g/100 g	6.01 ± 0.18[a]	6.07 ± 0.10[a]	6.37 ± 0.22[b]	6.46 ± 0.14[bc]	6.60 ± 0.16[c]	6.45 ± 0.24[bc]	6.38 ± 0.12[b]	*
Ash, g/100 g	0.51 ± 0.05[c]	0.44 ± 0.03[b]	0.38 ± 0.03[a]	0.37 ± 0.03[a]	0.35 ± 0.04[a]	0.36 ± 0.03[a]	0.37 ± 0.03[a]	*
TS, g/100 g	9.26 ± 0.81[ab]	8.94 ± 0.70[a]	9.62 ± 0.65[abc]	9.54 ± 0.46[abc]	9.69 ± 0.69[bc]	9.73 ± 0.54[bc]	9.93 ± 0.39[c]	*

[a–e]Means within a row without a common superscript letter are different ($P < 0.05$).

[1]Mean values ± SD.

*$P < 0.05$.

milk during the 180-d lactation period are shown in Table 2. Results were within the range of variability of data in the literature on donkey milk (Table 1). Various donkey breeds from different continents appear to produce milk very similar in composition, except for fat content (Table 1).

During lactation, the density of donkey milk remained constant at 1.032. This was lower than that reported by Mariani et al. (2001) for mare milk. The pH value, ranging from 7.14 to 7.22, did not vary significantly throughout the lactation period, which was consistent with the findings of Salimei et al. (2004). This suggests that the pH value was not influenced by breed or stage of lactation. The average pH value (7.18 ± 0.03) of donkey milk was higher than that of cow milk. This may be explained by the lower CN and phosphate contents in donkey milk than in cow milk (Salimei et al., 2004).

The protein content of donkey milk was similar to that of human milk and was much lower than that of cow milk (Table 1). Protein content varied significantly during lactation. Such a change in milk protein of the Jiangyue breed was rather smooth compared with protein variation in the Martina Franca and Ragusana breeds (Salimei et al., 2004). The total protein percentage continued to decline slowly to a minimum at d 120 PP and then increased until the end of lactation. Similar trends were observed in Haflinger mare milk (Mariani et al., 2001) and bovine milk (Fox, 2003). This showed that the total protein percentage in donkey milk was affected by the stage of lactation, consistent with the finding of Fox (2003) in bovine milk. This may be related to the differential expression of milk protein genes during lactation, as reported by Demmer et al. (1998) in the brushtail possum.

The average fat content of donkey milk was similar to that of mare milk and was much lower than in the milk of other mammals. The fat content of donkey milk reported in the literature (Table 1) showed marked variation, as also observed for mare milk (Table 1). A wide variability in fat content was also observed throughout the lactation in this study. The fat content of donkey milk was as low as 0.50% at d 15, after which it started to increase and peaked at 1.40% on d 105, followed by a sharp decline. A similar trend was reported by Salimei et al. (2004) for ass milk. In late lactation, the fat content increased again and reached 1.70 ± 0.32% on d 180. The fat content obtained in this study was consistent with the reports of some researchers (Anantakrishnan, 1941; Oftedal and Jenness, 1988; Taha and Kielwein, 1989) but was much higher than those reported by others (Salimei et al., 2004; Chiavari et al., 2005), indicating that fat content could be affected by breed, breeding area and forage, milking technique, and interval between milkings, as also reported by Fox (2003).

The lactose content of donkey milk was similar to that of human milk (Table 1) and was much higher than that of cow milk. The high lactose content obtained in this study was consistent with values reported by other researchers (Table 1). The concentration of lactose in donkey milk was nearly constant throughout the lactation, because lactose is responsible for about 50% of the osmotic pressure of milk, which is equal to that of blood. The decrease of lactose content in late lactation may be due to the influx of NaCl from the blood. The standard deviation in the test values from each group was so small that the lactose content in donkey milk seemed to be independent of breed, milking time, and stage of lactation, as observed by Salimei et al. (2004) for ass milk.

The ash content of donkey milk lay between the lower value of human milk and the higher value of cow milk (Table 1), consistent with other reports in the literature on donkey milk (Table 1). The ash content of donkey milk appeared to be unaffected by breed. There was a continuous decline in ash content from 0.51 ± 0.05 to 0.37 ± 0.03% during the lactation. The milk produced in the first month of lactation, when milk is the only nutritional source for the foal, contained the highest levels of mineral elements, which may be related to the

Table 3. Correlations between parameters[1] of donkey milk during the lactation

Item	Protein	Fat	Lactose	Ash	Total solids
pH	0.776*	–0.498	–0.820*	0.727	–0.553
Protein		–0.655	–0.986**	0.972**	–0.726
Fat			0.637	–0.785*	0.774*
Lactose				–0.940**	0.778*
Ash					–0.73

[1]Mean values in Table 2.

*$P < 0.05$; **$P < 0.01$.

considerable mineral requirements of the young foal at this fast growth stage. Afterward, the mineral supply of the milk decreased considerably (Doreau, 1994).

The TS content increased continuously during the entire lactation investigated. The variation in TS content corresponded to that of milk yield in early and middle lactation. In late lactation, the declining yield was accompanied by increases in TS and fat and a decline in lactose content. This finding agreed with the reports of Oftedal (1984, 1985).

The energy values of donkey milk at d 15, 30, 60, 105, 120, 150, and 180 of lactation were, on average, 405, 440, 462, 484, 455, 465, and 490 cal/kg, respectively. The variation in energy value appeared to show much similarity to that of the fat content.

Simple Linear Correlations

Simple linear correlations (Pearson's r) between parameters (mean values in Table 2) are reported in Table 3. The highest r values were recorded for the correlations between protein and lactose and between lactose and ash. However, these results must be validated under experimental conditions and the reasons for these parameters being so linearly correlated should be more thoroughly investigated.

Nitrogen Distribution

Changes in the nitrogen distribution of donkey milk protein fractions throughout the 180-d lactation period are shown in Table 4. Results were within the range of variability reported in the literature on donkey milk (Table 1) and showed remarkable differences compared with other mammalian milks. No significant differences were observed in the CNN content and percentage as lactation advanced. This may have been because the level of CN mRNA remained constant throughout the lactation, in accordance with the finding reported by Demmer et al. (1998) in the brushtail possum. The WPN and NPN contents showed a decreasing trend over the lactation. However, the concentrations of WPN and NPN as percentages of TN did not vary significantly throughout the lactation period and appeared not to be influenced by breed or lactation stage. The average percentages of CNN, WPN, and NPN were 52, 37, and 11%, respectively.

As shown in Table 1, there was a similarity between donkey milk and mare milk in nitrogen distribution. The whey protein content of donkey milk was close to that of human milk and the CN content was higher. The average ratio of CN to whey protein in donkey milk was intermediate between the lower value of human milk and the higher value of cow milk, consistent with the finding reported by Travia (1986). The high whey protein content of donkey milk makes it more favorable for human nutrition (Iacono et al., 1992; Curadi et al., 2001).

With reference to the protein content, one must not ignore the fact that patients who experience an intolerance to cow, goat, or sheep milk are able to tolerate donkey and mare milk (Gall et al., 1996; Businco et al., 2000; Carroccio et al., 2000; Curadi et al., 2001). Although the mechanism for tolerance to donkey milk is still unclear, it may be related to the specific levels of major allergenic components in the milk. Among the potentially allergenic milk components, β-LG is, in fact, probably the major milk allergen among infants and small children, whereas CN is considered the predominant milk allergen among adults (Carroccio et al., 1999). Lara-Villoslada et al. (2004) found that the balance between CN and whey protein played an important role in the sensitization capacity of cow milk and showed that cow milk with a CN:whey protein ratio of 40:60 was less allergenic than native cow milk. As a result, it may be of interest to use donkey milk for infant feeding. The donkey milk may be a valid substitute for human milk and also an alternative for feeding children who experience an allergy to cow milk.

SDS-PAGE and Densitometry

The SDS-PAGE patterns of donkey milk samples obtained on d 15, 30, 60, 90, 120, and 150 PP, respectively, and the human milk sample obtained on d 60 PP are shown in Figure 2. By recognition of the protein marker bands and by comparing protein migration patterns with those previously published for donkey and mare milk (Miranda et al., 2004; Salimei et al., 2004), it was possible to identify the following proteins: lactoferrin [approximate relative molecular mass ($\mathbf{M_r}$) of 75 kDa, serum albumin (approximate M_r of 60 kDa), CN (approximate M_r of 21 to 35 kDa), β-LG (approximate M_r of 18 kDa), lysozyme (approximate M_r of 15 kDa), and α-LA (approximate M_r of 14 kDa).

Quantitative determinations of the milk proteins were carried out by densitometry analysis and the data

Table 4. Nitrogen content (g/100 g) and percentage distribution of donkey milk protein fractions during the 180-d lactation period[1]

Protein fraction[2]	Lactation stage, d							
	15	30	60	105	120	150	180	*P*
CNN	1.02 ± 0.06	0.79 ± 0.07	0.78 ± 0.03	0.78 ± 0.13	0.79 ± 0.01	0.82 ± 0.09	0.77 ± 0.07	NS
WPN	0.70 ± 0.16[b]	0.70 ± 0.11[b]	0.58 ± 0.11[ab]	0.57 ± 0.11[ab]	0.47 ± 0.02[a]	0.45 ± 0.08[a]	0.59 ± 0.11[ab]	*
NPN	0.23 ± 0.03[c]	0.20 ± 0.03[b]	0.17 ± 0.01[a]	0.16 ± 0.02[a]	0.18 ± 0.01[ab]	0.16 ± 0.03[a]	0.15 ± 0.02[a]	*
CNN:TN	0.53 ± 0.03	0.47 ± 0.02	0.51 ± 0.03	0.51 ± 0.06	0.55 ± 0.01	0.57 ± 0.01	0.51 ± 0.06	NS
WPN:TN	0.35 ± 0.03	0.42 ± 0.02	0.38 ± 0.04	0.38 ± 0.07	0.33 ± 0.02	0.32 ± 0.02	0.39 ± 0.05	NS
NPN:TN	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.02	0.11 ± 0.02	0.12 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	NS

[a–c]Means within a row without a common superscript letter are different $P < 0.05$).

[1]Mean values ± SD.

[2]CNN = casein N; WPN = whey protein nitrogen; TN = total nitrogen.

*$P < 0.05$.

(expressed as the percentage of total protein) are presented in Table 5. Immunoglobulin must not be ignored, because according to a study carried out by Indian scientists, Ig in donkey milk can be a key to treating AIDS and tuberculosis (Anonymous, 1999). In our study, the Ig percentage increased gradually as the lactation progressed. The percentages of β-LG, lysozyme, and α-LA varied significantly; all continued to decrease to a minimum at d 90 or 120 PP and to increase thereafter. This may attributed to the changes in β-LG, lysozyme, and α-LA mRNA levels throughout lactation, with a transition in middle lactation as reported by Demmer et al. (1998) in the brushtail possum. In contrast, no significant differences were observed in lactoferrin, serum albumin, and CN percentages as lactation processed. The SDS-PAGE results agreed well with those of the nitrogen distribution analysis reported in this study. The change in whey protein content of donkey milk during the lactation could be attributed to the changes in β-LG, lysozyme, and α-LA.

By comparison with the SDS-PAGE patterns of human milk, the main features of donkey milk were the presence of β-LG and lysozyme at sufficiently high levels to be visualized, consistent with the reports of Civardi et al. (2002) and Salimei et al. (2004). However, β-LG was apparently absent from human milk, whereas Ig, lactoferrin, and serum albumin were present at high levels. In donkey milk, β-LG accounted for approximately 40% of the whey protein, equal to the level in mare milk and lower than that in bovine milk (Miranda et al., 2004). This finding may be related to the hypoallergenic characteristics reported for both donkey and mare milk (Businco et al., 2000; Carroccio et al., 2000; Curadi et al., 2001).

As shown in Table 5, by calculation, the percentage of lysozyme in donkey whey protein was about 25%, higher than in human and mare milk, whereas only a trace was observed in bovine milk (Solaroli et al., 1993; Miranda et al., 2004). Many authors have shown interest in the large amount of lysozyme in donkey milk, and according to Coppola et al. (2002), donkey milk represents an optimal growth medium for certain strains of useful lactic acid bacteria.

AA

There are no previous reports on the variation in AA content of donkey milk over a lactation. Our results

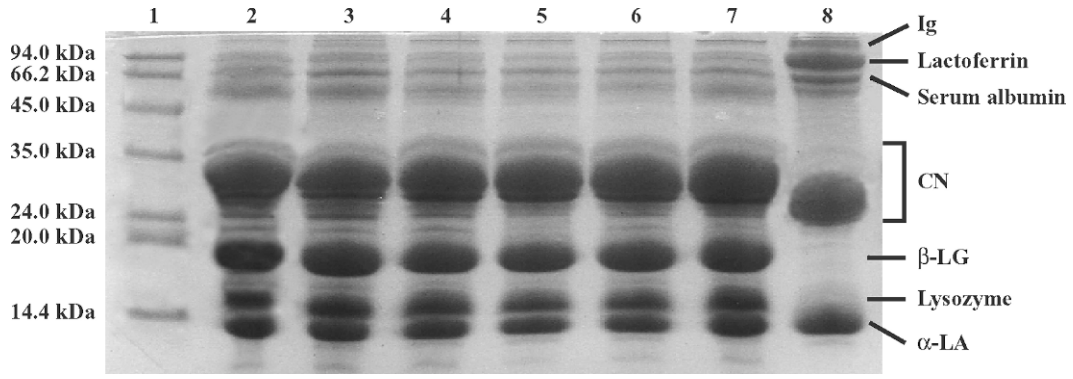

Figure 2. Sodium dodecyl sulfate-PAGE of donkey milk and human milk samples: Lane 1 is protein marker; lanes 2 to 7 are donkey milk samples obtained at d 15, 30, 60, 90, 120, and 150 postpartum, respectively; lane 8 is a human milk sample obtained at d 60 postpartum.

Table 5. Changes of protein components (% of total protein) in donkey milk during the 150-d lactation period and protein components of human milk obtained at d 60 postpartum[1]

Protein component, %	Lactation stage of donkey milk, d							
	15	30	60	90	120	150	P	Human milk
Ig	0.70 ± 0.16[a]	0.75 ± 0.14[a]	0.83 ± 0.03[a]	0.94 ± 0.18[a]	1.37 ± 0.33[b]	1.46 ± 0.14[b]	*	4.29 ± 0.54
Lactoferrin	2.13 ± 0.07	2.45 ± 0.38	2.02 ± 0.38	2.00 ± 0.27	2.12 ± 0.20	1.83 ± 0.37	NS	14.02 ± 1.55
Serum albumin	3.03 ± 0.36	2.81 ± 0.36	2.52 ± 0.41	2.98 ± 0.54	2.74 ± 0.23	2.33 ± 0.72	NS	7.37 ± 0.21
CN	38.09 ± 1.89	37.56 ± 1.48	39.98 ± 1.05	39.43 ± 1.92	39.37 ± 1.03	38.99 ± 1.55	NS	38.94 ± 1.83
β-LG	22.24 ± 0.76[ab]	23.27 ± 0.65[b]	21.78 ± 1.04[a]	22.13 ± 0.20[ab]	21.44 ± 1.08[a]	21.49 ± 0.83[a]	*	—
Lysozyme	13.91 ± 0.70[ab]	15.44 ± 0.29[b]	14.93 ± 0.88[b]	13.94 ± 0.63[ab]	13.13 ± 1.24[a]	14.48 ± 1.09[ab]	*	3.49 ± 0.56
α-LA	13.69 ± 0.23[b]	12.34 ± 0.74[a]	12.71 ± 0.26[a]	12.94 ± 0.14[ab]	12.23 ± 0.17[a]	12.54 ± 0.65[a]	*	25.82 ± 0.88
Whey protein	55.69 ± 1.44[ab]	57.06 ± 1.69[b]	54.79 ± 0.88[ab]	54.93 ± 0.95[ab]	53.03 ± 1.92[a]	54.13 ± 3.11[ab]	*	55.00 ± 1.49

[a,b]Means within a row without a common superscript letter are different ($P < 0.05$).

[1]Mean values ± SD.

*$P < 0.05$.

showed that the AA content did not vary significantly throughout the lactation period investigated, with the exception of Asp and Thr (Table 6). The AA content of donkey milk was not affected by stage of lactation. The contents of Asp and Thr declined continuously as the lactation advanced. However, we did not investigate the AA content in the first 5 d after foaling, when significant changes might have occurred.

Table 7 summarizes the AA composition of donkey, mare, and cow milk. Differences in the AA contents of different animal milks appear to be accompanied by differences in total protein contents. Compared with mare and cow milk, donkey milk had a lower AA content, probably because of its lower protein content. When the AA composition was expressed as grams of AA/100 g of protein, the differences were not so apparent. In addition, there were noticeably higher levels of Ser, Glu, Arg, and Val and a lower value of Cys in donkey milk. The percentages of 8 essential AA in the protein of donkey milk were higher than those of mare and cow milk. Taha and Kielwein (1990) reported that the mean values of most peptide-bound AA in donkey milk were higher than those of camel and buffalo milk. Thus, the AA composition of donkey milk also makes it more suitable for consumption by humans than is other mammalian milk.

CONCLUSIONS

Donkey milk was shown to be poor in protein and fat and rich in lactose, which is more similar to mare and human milk than to other mammalian milk. It was also

Table 6. Amino acid concentration (g of AA/100 g of milk) of donkey milk during the 180-d lactation period[1]

AA	Lactation stage, d							
	15	30	60	105	120	150	180	P
Asp	0.16 ± 0.02[b]	0.14 ± 0.01[ab]	0.16 ± 0.02[b]	0.15 ± 0.03[ab]	0.13 ± 0.03[ab]	0.16 ± 0.03[b]	0.12 ± 0.02[a]	*
Ser	0.10 ± 0.01	0.10 ± 0.04	0.11 ± 0.03	0.10 ± 0.04	0.08 ± 0.02	0.09 ± 0.02	0.09 ± 0.01	NS
Glu	0.40 ± 0.06	0.40 ± 0.07	0.39 ± 0.05	0.36 ± 0.07	0.30 ± 0.06	0.43 ± 0.05	0.33 ± 0.01	NS
Gly	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	NS
His	0.04 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	NS
Arg	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	0.08 ± 0.02	0.07 ± 0.00	NS
Thr	0.07 ± 0.01[b]	0.07 ± 0.01[b]	0.07 ± 0.01[b]	0.07 ± 0.02[b]	0.03 ± 0.01[a]	0.07 ± 0.03[b]	0.05 ± 0.00[ab]	*
Ala	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	NS
Pro	0.15 ± 0.03	0.16 ± 0.03	0.13 ± 0.05	0.14 ± 0.03	0.12 ± 0.03	0.16 ± 0.02	0.13 ± 0.00	NS
Cys	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	NS
Tyr	0.06 ± 0.02	0.07 ± 0.01	0.06 ± 0.02	0.06 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	NS
Val	0.12 ± 0.01	0.11 ± 0.02	0.11 ± 0.02	0.10 ± 0.03	0.09 ± 0.02	0.12 ± 0.02	0.10 ± 0.00	NS
Met	0.02 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	NS
Lys	0.13 ± 0.03	0.13 ± 0.02	0.13 ± 0.02	0.12 ± 0.02	0.10 ± 0.02	0.13 ± 0.02	0.11 ± 0.00	NS
Ile	0.10 ± 0.03	0.10 ± 0.02	0.10 ± 0.02	0.09 ± 0.02	0.07 ± 0.02	0.10 ± 0.02	0.08 ± 0.00	NS
Leu	0.14 ± 0.01	0.15 ± 0.02	0.15 ± 0.02	0.13 ± 0.02	0.12 ± 0.03	0.15 ± 0.02	0.13 ± 0.00	NS
Phe	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.09 ± 0.02	0.07 ± 0.00	NS

[a,b]Means within a row without a common superscript letter are different ($P < 0.05$).

[1]Mean ± SD.

*$P < 0.05$.

Table 7. Amino acid compositions of donkey, mare,[1] and cow milk, and donkey, mare, cow, and human milk proteins

	Milk, g of AA/100 g			Protein, g of AA/100 g			
AA	Donkey	Mare	Cow	Donkey	Mare	Cow	Human
Asp	0.140	0.246	0.26	8.9	10.4	7.8	8.3
Ser	0.098	0.147	0.16	6.2	6.2	4.8	5.1
Glu	0.358	0.474	0.77	22.8	20.1	23.2	17.8
Gly	0.019	0.045	0.06	1.2	1.9	1.8	2.6
His	0.036	0.056	0.1	2.3	2.4	3.0	2.3
Arg	0.072	0.123	0.11	4.6	5.2	3.3	4.0
Thr	0.056	0.101	0.15	3.6	4.3	4.5	4.6
Ala	0.055	0.076	0.10	3.5	3.2	3.0	4.0
Pro	0.138	0.197	0.32	8.8	8.4	9.6	8.6
Cys	0.007	0.014	0.02	0.4	0.6	0.6	1.7
Tyr	0.058	0.101	0.15	3.7	4.3	4.5	4.7
Val	0.102	0.097	0.16	6.5	4.1	4.8	6.0
Met	0.028	0.035	0.06	1.8	1.5	1.8	1.8
Lys	0.115	0.189	0.27	7.3	8.0	8.1	6.2
Ile	0.087	0.09	0.14	5.5	3.8	4.2	5.8
Leu	0.135	0.229	0.29	8.6	9.7	8.7	10.1
Phe	0.068	0.111	0.16	4.3	4.7	4.8	4.4
Try	—	0.028	0.05	—	1.2	1.5	1.8
Essential AA	0.600	0.866	1.25	38.2	36.7	37.5	40.7
Total	1.572	2.359	3.33	100	100	100	99.8

[1]Csapó-Kiss et al. (1995).

characterized by a low CN content and a particularly a high whey protein content that was rich in β-LG and lysozyme. The percentages of 8 essential AA in the protein of donkey milk were higher than those of mare and cow milk; the milk also had higher levels of Ser, Glu, Arg, and Val and a lower level of Cys. As a result, donkey milk exhibited unique nutritional characteristics and has optimal potential to be used as a new dietetic food and breast milk substitute.

Lactation stage affected the gross composition of donkey milk but had no significant effect on pH, percentage of whey protein and CN, and AA content, except Asp and Thr. The work may be a promising basis for the study and exploitation of donkey milk. However, more systematic studies are needed.

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