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Full Length Research Paper

Comparison of the biochemical components and characteristic of milk between Tibetan sheep and goat in neighboring area

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The nutrient composition, enzyme activities, the profile of free amino acids (FAAs) and protein compositions in Tibetan sheep milk were assayed and compared with those in Chengdu Ma goat. The results showed that the total solids, milk fat and activity of alkaline phosphatase (AKP) in Tibetan sheep milk were significantly higher than that in Chengdu Ma goat. Conversely, the activities of γ-glutamyltransferase (γ-GT) and lactoperoxidase (LP) were significantly lower than that in Chengdu Ma goat. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) results showed that the concentration of casein (CN) in Tibetan sheep milk (accounting for more than 50% of total milk protein) was significantly lower than that in Chengdu Ma goat, but the concentration of β-lactoglobulin (β-Lg) and serum albumin (SA) were significantly higher than that in Chengdu Ma goat. Seventeen different FAAs were identified from the Tibetan sheep milk by reversed phase high performance liquid chromatography (RP-HPLC). Arg was the most abundant FAA (192.15 ± 119.78 mg/L), while Ala was the poorest one (4.34 ± 1.12 mg/L). Interestingly, the concentrations of the following amino acids: Asp, Gly, Lys, Glu, Thr, Ala and Val in Tibetan sheep milk were significantly lower than those of Chengdu Ma goat. On the contrary, the Met concentration of Tibetan sheep was significantly higher than that of Chengdu Ma goat. The distinct nutritional characteristics of Tibetan sheep milk indicated their adaption to the harsh environment in Tibetan area.

Key words: Tibetan sheep, milk composition, free amino acids (FAAs).

INTRODUCTION

The reason why goat and sheep milk is considered as a food of exceptional quality is due to the complete essential amino acids, adequate mineral supply, similar mass fraction of lactose with human milk, small fat globule it provides and especially its ease to absorb (Klinger and Rosenthal, 1997). However, information on the composition of Tibetan sheep milk is extremely limited, the content of total solids, protein, lactose, free amino acids (FAAs) and the activity of milk enzyme was not being detected. The quantitative and qualitative characteristics of FAA of milk of different mammalian species are distinctive, which may reflect different nutritional needs of FAA during early postnatal development among different species (Davis et al., 1994; Sarwar, 2001, Sarwar et al., 1998). In order to increase the amount of FAA and adjust the relative proportions to resemble that of human milk, researchers should not only clarify the FAA content of human milk but also increase our knowledge of FAA content of milk of other mammalian species.

Tibetan sheep (*Ovis Aries*) is a remarkable animal developed on the Qinghai-Tibetan Plateau often called the “roof of the word” and as the most extensive high-

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Abbreviations: FAAs, Free amino acids; **AKP,** alkaline phosphatase; **γ-GT,** γ-glutamyltransferase; **LP,** lactoperoxidase; **AMY,** amylase; **SDS-PAGE,** sodium dodecyl sulfate polyacrylamide gel electrophoresis; **RP-HPLC,** reversed phase high performance liquid chromatography; **CN,** casein; **β-Lg,** β-lactoglobulin; **SA,** serum albumin; **α-La,** α-lactalbumin.

elevation region on the earth. As one of the famous coarse hair sheep breed, it can adapt to life on the plateau and many adaptability changes may influence its milk composition. Although, the nutritional value of sheep and goat milk have been thoroughly studied during the past decades. These kinds of study related to the milk composition, characteristic of cheese, milk protein polymorphism, mammary gland health and so on (Fuertes et al., 1998; Jaeggi et al., 2003; Pirisi et al., 1999; Ferranti et al., 1995; Chianese et al., 1996; Bianchi et al., 2004). However, there are no comprehensive studies to characterize the biochemical composition in Tibetan sheep milk although it was a potential milk resource for the people living in this area. Here we report the determination of nutrient composition, enzyme activities, free amino acids of Tibetan sheep milk and its characteristics and the results were compared to the milk component of Chengdu Ma goat. Our aim is to lay a solid basis for further study involved in controlling milk yield and composition and improving ewe and newly born lambs management of Tibetan sheep.

MATERIALS AND METHODS

Animal and milk sample

Milk sample were collected from 63 healthy Tibetan ewes after parturition (3rd month) at Ruoergai county farm in Tibetan plateau on March 2005. A preliminary examination and serological evaluation were conducted to ensure that the experimental animals were in a healthy condition. All ewes were grazing on the natural pasture. The average age of selected ewe is 4 years old and parities are from 1 to 4. In order to compare the milk contents with Tibetan sheep, 34 milk samples from Chengdu Ma goat were also collected at Qionglai County farm near Tibetan plateau.

Collection of milk sample and pre-processing

Each ewe was milked manually every morning (8:00 - 11:00 am), 8 ~ 10 mL mixed milk sample were collected from each ewe. Milk samples were frozen immediately after collection at each milking and stored at 70℃ until analysis. For normal nutritional analysis, we used the thawed milk sample directly. For enzyme activity and FAA contents detection, the thawed milk sample were centrifuged at 800 g, 4℃ for 20 min, then the down phase were divided into 6 then put into 1.5 ml EP tube. Four out of the 6 were stored in -20℃ for late enzyme activity detection.

Instrument and reagent

Instruments

The main instruments include: 1100 High Pressure/Performance Liquid Chromatography (Agilent) Cary 50 ultraviolet spectrophotometer (Varian) Gel Doc 2000 (Bio-Rad) Freeze Drying Equipment (Servant) BP211D electronic balance (Sartorius), Mi2 cromax Centrifuge (ICE), MilliQ (Millipope), PHS-2C meter (Jing Ke Lei V Chi).

Chemicals and reagents

For normal nutritional analysis and enzyme activity detection, the following were used: Acrylamide (Sigma,) Standard Protein Marker (Promega), SDS (Gibicobrl), 4-NPP/4-Nironhenyl phosphate disodium salt (AVOCADO), o-phenylendiaminedihydrochloride (Sigma), Triton X-100 (MERCK), methyl cyanide, methyl alcohol, tetrahydrofuran were chromatographic grade (TEDIA). For Determination of FAA contents, mobile phase A: 20 mmoL/L sodium acetate + 0.018% massfraction Triethylamine + 1.5 mL tetrahydrofuryl alcohol, fill the volumetric flask to 1 L, pH (7.20 ± 0.05) Mobile phase B: 20 mmoL/L Sodium acetate, methyl cyanide: methyl alcohol (V:V:V = 1:2:2, pH (7.20 ± 0.05); methyl cyanide, methyl alcohol, tetrahydrofuran were chromatographic grade (TEDIA), standard amino acids (10, 25, 100, 250 pmoL, 1 nmoL/μL, Agilent) were used. The remaining reagents were analytically pure.

Analysis of milk sample

Determination of nutrition component of Tibetan milk

The purified protein concentration was determined by Bradford method as described by Bradford (1976). Lactose concentration was measured by Chelatocolorimetry Colorimetry (Teles et al., 1978). Content of milk fat was detected by a simple UV spectrophotometric method (Forcato et al., 2005).

A total of 40 milk samples from different lactations (18 Tibetan milk samples from the 1st lactation, 13 samples from the 2nd lactation and 9 samples from the 3rd - 4th lactation) were collected for milk enzyme activity detection. Gamma-glutamyltranspeptidase (γ-GT) activity was assayed by using the color reaction of a ferrous salt complex and a primary aromatic amine (Minato, 1979). Alkaline phosphatase (AKP) activity was measured by a spectrometry method using p-nitrophenylphosphate as substrate (Kawade, 1964). Lactoperoxidase (LP) activity was measured as described by Hurley (1987). Amylase (AMY) activity was detected by spectrometry method based on the reaction of starch and iodine (Van loon et al., 1952). Then the specific activity of each enzyme was determined according to their activity and content.

Determination of milk protein contents

The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 15% (v/v) separating gel according the method established by Laemmli (1970) and the protein were migrated under β-mercaptoethanol, then were visualized by Coomassie Brilliant Blue R-250 staining. The result was recorded by GDS-800 and then the protein bands and relative concentration were detected.

Determination of FAA contents

The concentration of FAA in skim milk samples from 40 random selected Tibetan sheep and all samples of Chengdu Ma goat were measured by reversed phase high performance liquid chromatography (RP-HPLC) method (Agilent 1100). The main steps were performed briefly. For the pretreatment of skim milk samples, 100 μL of skim milk were added into a 2 mL centrifuge tube, then 4 fold of ethanol was also added, after that, the tubes were put into 4℃ for 30 min. After spinng, the tubes were taken on a vortex mixer and then centrifuge at 1,500 g, 4℃ for 20 min. The upper phase was filtered by 0.45 μm membrane filter. For the RP-HPLC parameters, the pre-column derivation of amino acid and RP-HPLC were auto performed by prestored program in RP-HPLC apparatus. Briefly, 5.0 μL boracic acid buffer was injected, and then, 1.0 μL OPA, the probe was wash using ddH_2O and 2.0 μL sample injected. Probe was washed again by ddH_2O and the contents mix 6 times *in situ*. 1.0 μL Fluorenylmethyloxycarbonyl chloride (FMOC) was

Table 1. Eluted gradient of mobile phase.

List	Time/min	Mobile phase A/%	Mobile phase B/%	Velocity/mL·min^{-1}
1	0.00	100.0	0.0	0.450
2	17.00	40.0	60.0	0.450
3	18.00	0.0	100.0	0.450
4	18.10	0.0	100.0	0.450
5	18.50	0.0	100.0	0.800
6	23.90	0.0	100.0	0.800
7	24.00	0.0	100.0	0.800
8	25.00	100.0	0.0	0.450

Table 2. The content of regular nutrient composition in Tibetan sheep milk (g/L).

Nutrient component	1st lactation	2nd lactation	3rd lactation	4th lactation	Mean
Protein	$44.83^{aA} \pm 10.12$	$51.83^{b} \pm 12.57$	49.64 ± 13.68	$56.75^{B} \pm 14.41$	48.45 ± 13.21
Lactose	41.51 ± 4.01	41.95 ± 5.45	43.17 ± 4.00	42.31 ± 4.84	41.93 ± 5.06
Fat	$70.86^{a} \pm 11.02$	68.14 ± 13.41	66.72 ± 12.10	$64.54^{b} \pm 8.77$	69.43 ± 11.41

Values with different superscript small letters show significant difference between two lactations (p<0.05), and the different superscript capitalized letters show extreme difference between two lactations (p < 0.01).

Table 3. The comparison of regular nutrient composition between Tibetan sheep and Chengdu Ma goat (g/L).

Breed	N	Total Solid	Protein	Lactose	Fat
Tibetan sheep	63	$169.45^{A} \pm 7.52$	48.45 ±13.21	41.93 ± 5.06	$69.43^{A} \pm 11.41$
Chengdu Ma goat	34	$157.88^{B} \pm 2.16$	44.18 ± 2.65	46.44 ± 3.06	$58.69^{B} \pm 2.71$

Injected, the probe washed using ddH_2O again, the contents mix 3 times *in situ* and 9.0 µL samples injected. For the separation condition, Hypersil ODSC1 (2.1 × 200 mm) analytic column were kept at 40°C, the flow-rate was 0.45 mL·min^{-1}. The detection wavelength is 338 nm and then adjusted to 262 nm at 16 min. The details of gradient elution steps are listed in Table 1.

Statistic analysis

Mean and standard deviation were calculated by Excel software. The statistic analysis of general nutrition contents, milk enzyme activity, the proportion of milk protein contents, the difference of FAA contents and proportion between Tibetan sheep and Chengdu Ma goat and different lactation times were calculated by T-test and general linear model.

RESULTS

The nutritional composition in Tibetan sheep milk

The nutritional composition of Tibetan sheep milk was detected in this study. The results showed that the concentration of milk protein, lactose and fat of Tibetan sheep were 48.45 ± 13.21, 41.93 ± 5.06 and 69.43 ± 11.41 g/L), respectively. The nutritional composition at different lactation is shown in Table 2. The results showed the correlation among milk protein concentration and different lactations. Significant difference was found between the 2nd and 1st lactation (p < 0.05); milk protein concentration of the 4th lactation is significantly higher than the 1st lactation (p < 0.01). But no significant difference of the lactose concentration was found among different lactation (p > 0.05). Interestingly, fat contents decreased when the lactation number increased, and the fat contents of the 1st lactation is significantly higher than that of the 4th lactation (p < 0.05).

A comparison of milk protein contents between Tibetan sheep and Chengdu Ma goat is shown in Table 3. The result indicated that milk solid substance of Tibetan sheep is higher than that in Chengdu Ma goat (exceeding 11.57 g/L), the difference between them was significant; but no significant difference were detected between Tibetan sheep and Chengdu Ma goat in its protein and lactose concentration (p < 0.05).

Milk enzyme activity and specific activity

The detection of milk enzyme activity and specific activity indicated that the average enzyme activity of γ-GT, AKP, LP, AMY were (2681.16 ± 911.68) U/L, (2207.59 ± 970.59) U/L, (3750.25 ± 3213.57 and (739.72 ± 558.01

Table 4. Enzyme activity and ratio activity in Tibetan sheep milk (U/L).

Lactation	γ-GT		AKP		LP		AMY	
	Activity	R. activity	Activity	R. activity	Activity	R. activity	Activity	R. activity
1 (n = 18)	2698.61 ± 715.72	7.32 ± 2.97	2626.22 ± 981.51	7.10 ± 3.36	4660.13 ± 3930.24	130.02 ± 110.23	896.42^{a} ± 728.41	2.32^{aA} ± 1.91
2 (n = 13)	2655.74 ± 904.92	5.58 ± 2.49	2375.43 ± 1149.12	4.95 ± 2.65	3910.21 ± 4150.32	80.25 ± 90.17	1055.43^{A} ± 812.01	2.09^{A} ± 1.52
3 – 4 (n = 9)	2689.13 ± 1114.41	5.90 ± 3.11	1621.13 ± 781.14	3.42 ± 1.81	2680.41 ± 1560.15	60.41 ± 40.12	267.30^{bB} ± 133.62	0.58^{bB} ± 0.30

Units of all enzyme activity are U/L, and units of all enzyme ratio activity are $\times 10^{-2}$U/mg protein besides LP being U/mg.

U/L), respectively; the average specific activity of them were (6.27 ± 2.86 × 10^{-2}, 5.17 ± 2.61 × 10^{-2}, 90.23 ± 80.17 and 1.66 ± 1.24 × 10^{-2} U/mg^{-1}) protein, respectively. The results also showed that significant differences were detected in 4 kinds of milk enzymes from different lactation time. Enzyme activity of γ-Gt ranged from 1035.12 to 5071.21 U/L; LP ranged from 60.02 to 4000.30 U/L, one of them is up to 177300.01 U/L (Table 4).

The average enzyme activity and specific activity of different lactation in Tibetan sheep are shown in Table 4. The Amy activity of the 3rd and 4th lactation is significantly lower than the samples collected from the 1st and the 2nd lactation ($p < 0.05$, $p < 0.01$); but no difference was found among γ-GT enzyme activity among different lactations ($p > 0.05$); faint differences were found among AKP and LP enzyme activity among different lactation, but the difference was not obvious ($p > 0.05$).

The comparison of the milk enzyme activity between Tibetan sheep and Chengdu Ma goat are shown in Table 5. The results revealed that the enzyme activity of γ-GT is significantly higher than that in Chengdu Ma goat ($p < 0.01$) and the enzyme activity of AKP and LP was significantly lower than that in Chengdu Ma goat ($p < 0.01$). No difference of AMY enzyme activity was found between these two species ($p > 0.05$). We can also found from Table 5, there were significant difference among the 4 kinds of milk enzyme among different individual.

Detection of protein compositions

SDS-PAGE of skim milk sample of Tibetan sheep and Chengdu Ma goat were conducted under reduction condition to ensure the proteins were separated according to size. The clear protein bands of α-lactalbumin (α-La), β-lactoglobulin (β-Lg), IgG-L, casein (CN), IgG-H, serum albumin (SA) were visualized by Coomassie Brilliant Blue R-250 staining (Figure 1).

The milk protein content ratios were identified by scan method. Table 6, shows the relative contents of protein components in Tibetan sheep milk**.** The ratio of CN in all samples exceeded 50%, ranged from 50.30 ± 10.19 to 52.09 ± 10.11%. The ratio of β-Lg was 20% and ranged from 19.24 ± 5.01 to 20.69 ± 5.43%; the lowest one was α-La, it ranged from 3.62 ± 1.23 to 4.62 ± 1.13% and its ratio varied among individuals. The ratio of β-Lg / α-La was 4.58. However, no significant difference was indentified among the ratio of same protein of Tibetan sheep milk sample at different lactation ($p > 0.05$).

We also compared the protein ratio of Tibetan sheep with Chengdu Ma goat milk sample (Table 7). The result indicated that the β-Lg and SA ratio of Tibetan sheep milk was significantly higher than that in Chengdu Ma sheep ($p < 0.05$ and $p < 0.01$, respectively); meanwhile, the CN ratio of Tibetan sheep was significantly lower than that in Chengdu Ma goat ($p < 0.01$). However, no difference was found for the ratio of α-La and IgG ($p > 0.05$).

Detection the proportion of FAA

Reproducibility and recovery of analysis method

The proportion of 17 amino acids showed that the co-efficient of variance of the proportion of FAA is less than 10% which implied that the instrument was reliable. The standard amino chromatograms (1 mmol/μL) and the detected amino acid chromatograms are shown in Figures 2 and 3, respectively. The correlation coefficient between the peak value and the concentration of 17 amino acids ranged from 0.995 to 0.999 while the concentration of amino acids ranged from 0.01 to 1 mmol/L. Equal amount of standard amino acids (194.70 mg/L) were added into the 4 milk samples (2 for Tibetan sheep and 2 for Chengdu Magoat). Seventeen FAAs were identified in milk of both Tibetan sheep and Chengdu Ma goat. Among the 17 FAAs detected in Tibetan sheep milk, the most abundant one was Arg (192.15 ± 119.78 mg·/L). The others following arginine in quality marks were Asp, Glu, Lys and Met. The lowest is Ala (4.34 ± 1.12 mg/L). The proportion of essential

Table 5. The comparison of enzyme activity in milk between Tibetan sheep and Chengdu Ma goat (U/L).

Breed	N	γ-GT	AKP	LP	AMY
Tibetan sheep	40	2681.16 ^{A} ± 911.68	2207.59 ^{B} ± 970.59	3750.25 ^{B} ± 3213.57	739.72 ± 558.01
Chengdu Ma goat	34	1942.74 ^{B} ± 943.32	3055.44 ^{A} ± 1251.21	5450.12 ^{A} ± 2810.34	827.33 ± 696.72

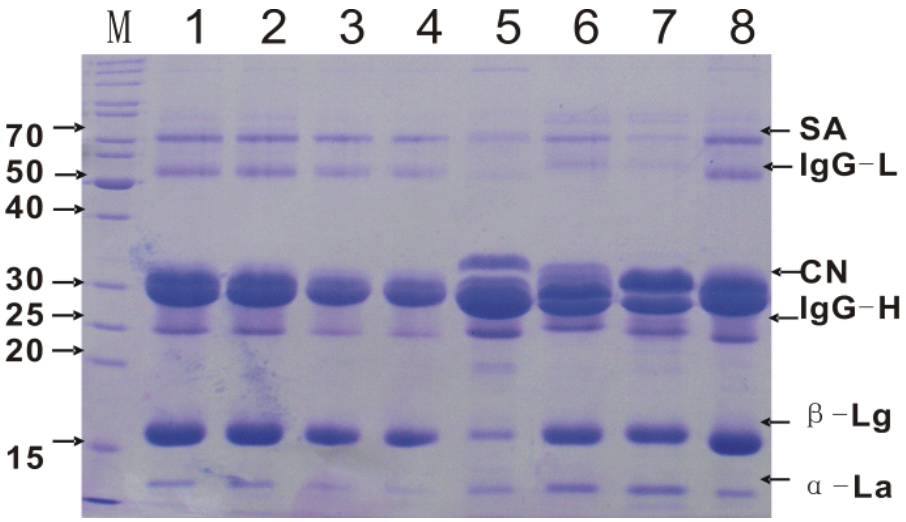

Figure 1. Electrophoresis photograph of SDS-PAGE of skim milk.
Lane 1:Protein marker kD; Lane 2 ~ 5: Tibetan sheep; Lane 6: Chengdu Ma goat; Lane 7: yak; Lane 8: Chinese Holstein-Friesian.

Table 6. Relative contents of protein components in Tibetan sheep milk (%).

Component	1st lactation	2nd lactation	3rd lactation	4th lactation	Mean
α-La	4.62 ± 1.00	3.62 ± 1.23	4.43 ± 1.14	4.62 ± 1.13	4.42 ± 1.18
β-Lg	20.69 ± 5.43	19.24 ± 5.01	20.50 ± 4.98	20.60 ± 4.99	20.23 ± 5.07
IgG-L	10.03 ± 2.94	9.61 ± 2.96	10.21 ± 2.84	10.17 ± 2.84	10.06 ± 2.90
CN	52.09 ± 10.11	52.02 ± 10.16	50.30 ± 10.19	50.53 ± 10.11	51.06 ± 10.11
IgG-H	6.36 ± 3.21	6.24 ± 3.34	6.73 ± 3.41	6.47 ± 3.20	6.42 ± 3.49
SA	6.03 ± 2.16	6.73 ± 2.11	6.75 ± 2.18	6.00 ± 1.99	6.38 ± 2.24

Table 7. The comparison of protein components in milk between Tibetan sheep and Chengdu Ma goat (%).

Breed	N	α–La	β–Lg	IgG	CN	SA
Tibetan sheep	63	4.42 ± 1.18	20.37 ^{a} ± 5.07	8.63 ± 3.10	51.06 ^{B} ± 10.11	6.38 ^{A} ± 2.24
Chengdu Ma goat	34	5.80 ± 0.61	14.77 ^{b} ± 2.06	7.31 ± 0.83	70.04 ^{A} ± 2.55	2.08 ^{B} ± 0.23

amino acids is 23.64%, accounts for 122.18 ± 34.18 mg/L. The Met concentration of Tibetan sheep milk was significantly higher than that in Chengdu Ma goat ($p < 0.01$), however the concentration of Asp, Gly, Lys, Glu, Thr, Ala and Val of Tibetan sheep milk samples weresignificantly lower than those of Chen-gdu Ma goat ($p < 0.05$). However, the total concentration of FAA was similar in milk of these two species ($p > 0.05$), meanwhile no difference of the total concentration of FAA among different lactations were found ($p > 0.05$). The proportion of FAA of Tibetan sheep and Chengdu Ma goat are shown in Table 8.

DISCUSSION

Detection of general nutrient compositions of Tibetan sheep milk showed that the total solids and fat of Tibetan

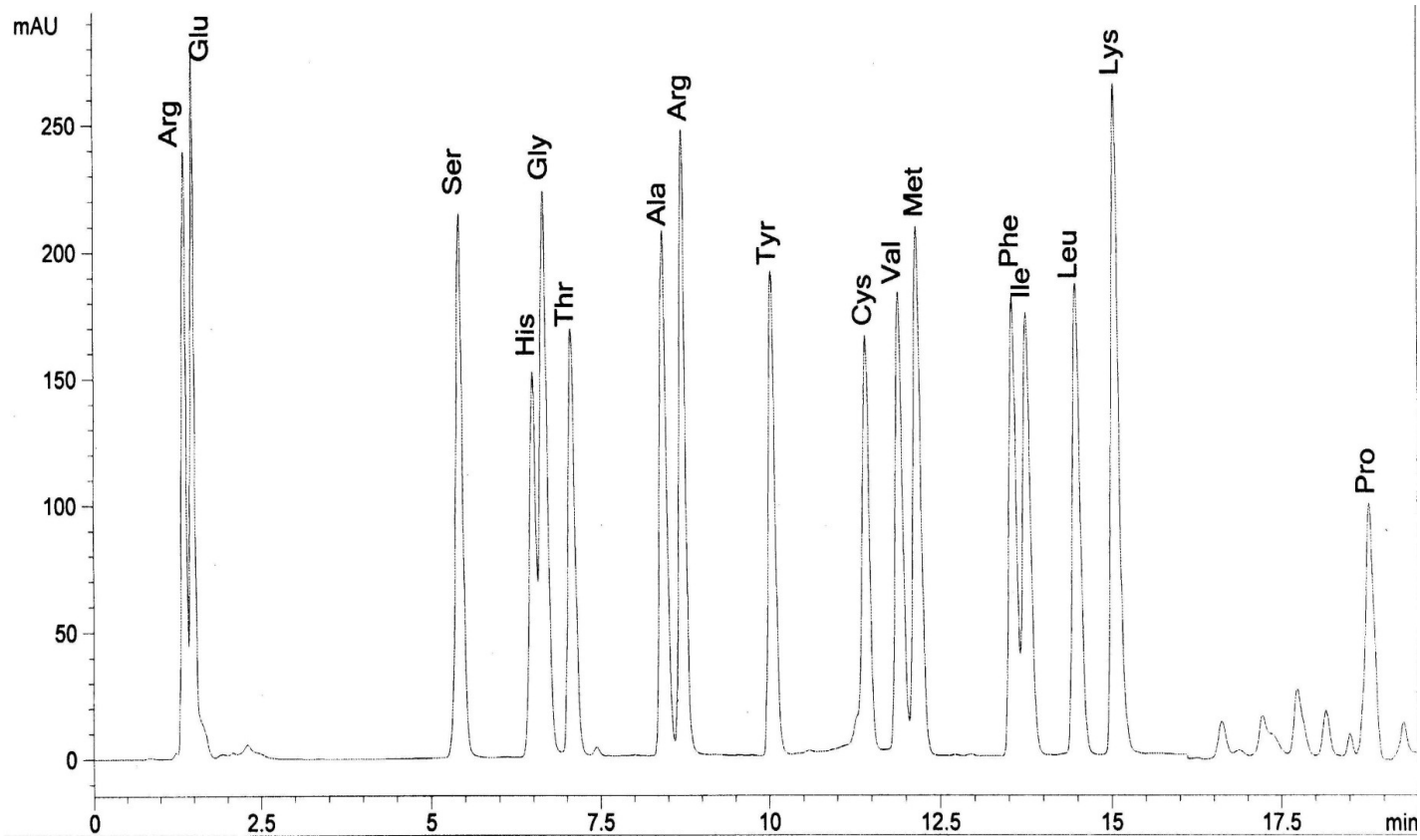

Figure 2. RP-HPLC profile of standard amino acids (T·min-1). VWD1 A, Wavelenght = 338 nm, TT(MAWEI\1NMOL.D).

Table 8. Quality mark of FAA in Tibetan sheep and Chengdu Ma goat (mg/L).

FAAs	Tibetan sheep (n = 40)	Chengdu Ma goat (n = 30)
Asp	38.05 ± 15.86	71.78 ± 17.96 *
Glu	36.73 ± 10.06	68.44 ± 8.43 **
Ser	5.87 ± 2.57	10.45 ± 7.37
His	38.05 ± 8.92	45.91 ± 9.28
Gly	6.34 ± 2.24	13.65 ± 2.89*
Thr	23.12 ± 9.47	44.71 ± 7.00**
Ala	4.34 ± 1.12	33.83 ± 1.33**
Arg	192.15 ± 119.78	219.71 ± 82.05
Tyr	9.61 ± 3.99	10.61 ± 6.90
Cys-SS-Cys	10.67 ± 3.90	9.15 ± 3.24
Val	17.54 ± 6.79	35.39 ± 8.16**
Met	31.05 ± 11.29**	18.23 ± 9.16
Phe	7.87 ± 4.50	10.00 ± 5.10
Ile	4.91 ± 1.21	5.61 ± 2.62
Leu	16.53 ± 4.06	19.61 ± 5.32
Lys	34.28 ± 2.33	37.73 ± 3.67 *
Pro	29.72 ± 3.97	34.66 ± 4.12
Essential AA	122.18 ± 34.18	126.57 ± 34.03
Nonessential AA	394.66 ± 108.97	562.90 ± 117.45
Total	516.84 ± 129.13	689.47 ± 103.32

*p < 0.05; ** p<0.01; Essential amino acid including Val, Met, Phe, Ile, Leu, Lys.

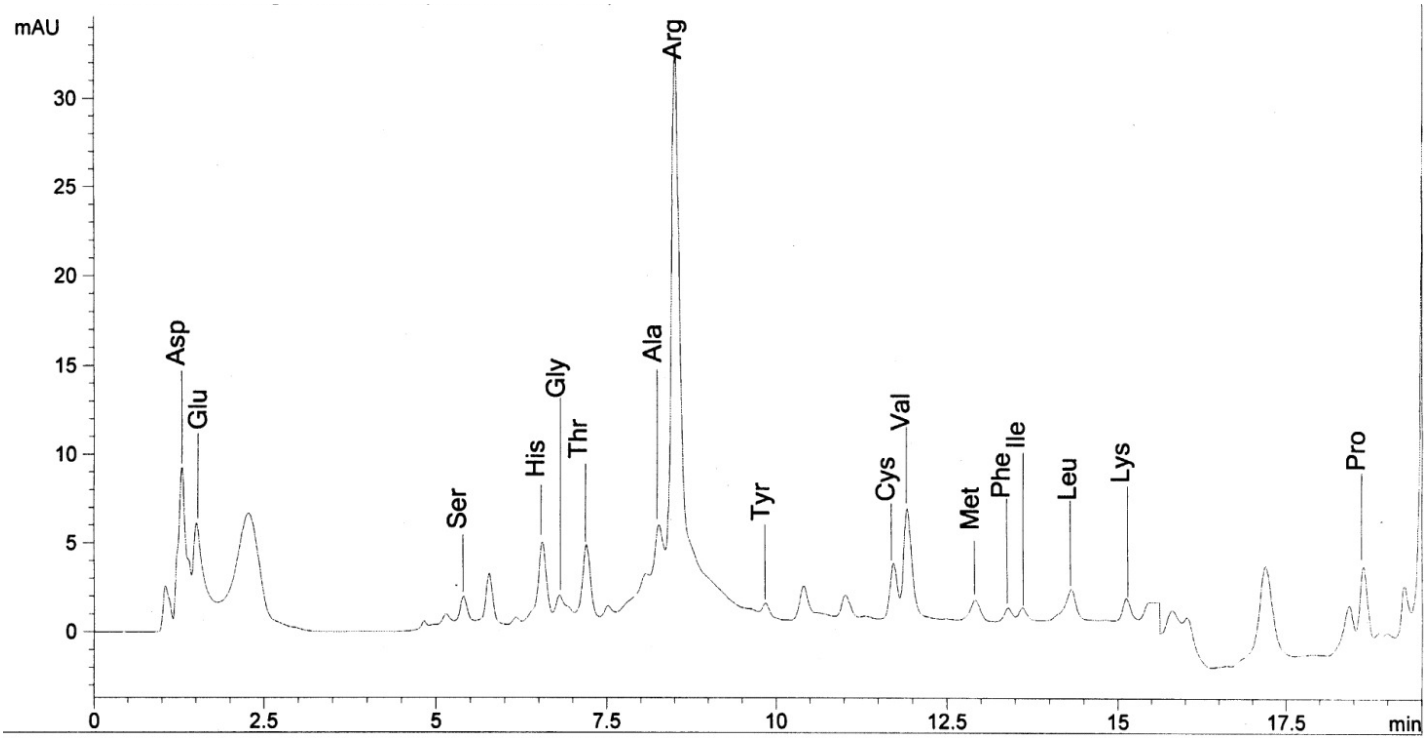

Figure 3. RP-HPLC profile of amino acids in Tibetan sheep milk (T·min-1). VWD1 A, Wavelenght = 338 nm, TT(MAWEI\SHEEP1.D).

sheep milk are significantly higher than that in Chengdu Ma goat ($p < 0.01$). Our results are consistent with the report by De la Fuente et al., (1997). The protein is slightly lower than De la Fuente et al., (1997) report but almost the same fat concentration is reported in sheep milk ($p > 0.05$). The milk yield of Tibetan sheep was lower than that in Chengdu Ma goat and some of the normal sheep. However, the high concentration of milk fat and other nutrition contents can ensure the development of the new born kidlet. The result implies that abundant milk fat and the total solids is an important characteristic of Tibetan sheep milk.

During the past decades, a lot of enzymes were identified in animal milk (Jennse, 1985; Katsoulos et al., 2010), most of them are synthesized in the epithelial cell of mammary gland and secreted by the epithelial cell (Shahani et al., 1973, 1980), and part of the enzymes are from blood (Shahani et al., 1980). Milk enzyme can influence the function and health of mammary gland, furthermore it also affect the development of new born lamb (Hartmann et al., 1989). The enzyme activity changes of these enzymes can reflect the function of the mammary gland tissue. Both milk AKP and milk γ-GT were bound to cell membrane, most of them distribute on milk fat globule membrane and secreted along with milk fat globule (Baumrucker and Pocius, 1979). Lp belong to the anti-bacterial dioxide system (LPS), it can reduce the number of bacterial of mammary gland, keep milk fresh, and decrease or prevent the diarrhea of new born lamb (Reiter et al., 1976).The γ-GT activity of Tibetan sheep is significantly higher than that in Chengdu Ma goat ($p < 0.01$), but the AKP activity is significantly lower than that in Chengdu Ma goat ($p < 0.01$); it reflect the different milk characteristics among different species. Meanwhile, the activity of AKP of Tibetan sheep milk is significantly higher than that in yak that distribute in the same area ($p < 0.01$), but the activity of γ-GT and LP are significantly lower than that in yak ($p < 0.01$) (results not showed). Now, the fawn survival rate of Tibetan sheep lamb is around 60%, this mainly due to hard and specific environment of Tibetan plateau, but the low LP activity implied that we would better prevent the diarrhea and disease of new born lamb and this may increase the fawn survival rate.

The main protein in Tibetan sheep milk is casein (50%), although it still lower than the casein concentration in cow and goat milk (70%). We compared the milk protein of Chengdu Ma goat and Tibetan sheep, the results showed that casein in Tibetan sheep milk is significantly lower than that in Chengdu Ma goat ($p < 0.01$). Due to the low content of α-La in Tibetan sheep milk, the ratio of β-Lg/α-La is 4.58, and is obviously higher than the ratio in milk of goat (ranged from 2 - 3) (Wang et al., 1995). The difference of the milk protein contents in Tibetan sheep milk can partially be attributed to the environment, feeding and management condition during lambing season (Thomas et al., 2001).

The most abundant free amino acid is Arg in milk of Tibetan sheep (192.15 ± 119.78 mg/L). Arg is not an essential amino acid for the adult Tibetan sheep, but for

the growing lamb, they need more Arg from food because they cannot synthesize enough Arg by themselves (Wu et al., 2000). Previous studies showed that the concentration of Arg in cow and human milk is 2.2 and 10.6 mg/L, respectively (Agarwal et al., 1975). The high concentration of Arg in Tibetan sheep milk may reflect the nutritional needs of the new born lamb. The high concentration of Arg in Tibetan sheep milk may play an important role to the development of new born lamb and reflect the adaptive changes of Tibetan sheep to the high elevation, cold and anoxic environment. Our results also showed that the Met concentration in Tibetan sheep milk was significantly higher than that in Chengdu Ma goat ($p < 0.01$). Met can bind with ATP and produce S-adenosyl-L-methionine *in vivo* and is involved in the physiological function of liver and fat transfer *in vivo* (Wang et al., 2001). Met can also be used as treatment for human depression (Williams et al., 2005). The high concentration of Arg and Met implies the high nutritional value of Tibetan sheep milk which means it can be used in health care. It also suggests that Tibetan sheep can be treated as a potential animal to produce high quality milk.

Our determination of FAA is consistent with the results of previous researches. The FAA of Tibetan sheep and Chengdu Ma goat milk is lower than that in human milk (976 mg/L) and other primates (Sarwar et al., 1998; Chuang et al., 2005). Attentions should be attracted to the feedstuff condition of these two breeds when we compare the FAA concentration of milk of Tibetan sheep and Chengdu Ma goat. Chengdu Ma goat was fed under a mild climate and abundant fodder was supplied during the lambing season. Meanwhile, Tibetan sheep distribute in Tibet plateau famous for its high altitude, cold and anoxic environment, and lack of fodder during the lambing season. Interestingly, although the total FAA and the mass fraction of many FAA in Tibetan sheep milk are lower than that in Chengdu Ma goat, the concentration of essential amino acid are similar ($p > 0.05$).

Conclusion

Detection of general nutrient compositions, milk enzyme activity, the protein composition and profile of free amino acids (FAAs) showed the milk of Tibetan sheep has distinct characteristics. The difference of the milk protein contents in Tibetan sheep milk with other species can partially be attributed to the milk of different species having its own characteristics, but it may due to the environmental factors (feeding and management condition in this area), especially the feeding condition during the lambing season. Distinct characteristics of its milk means Tibetan sheep can be used to produce high quality milk. The results can also be used to improve the feeding and management of ewe during the lambing season, increase the protein content of its feed; through these ways, the growth of new born lamb can be ensured.

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