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Chemical Compositions and Nutrients Profiling of Yak Milk in Chinese Qinghai-Tibetan Plateau

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Abstract: Yak in the Qinghai-Tibetan Plateau depends mainly on grazing and milk is one of its main products. In this study, the compositions of yak milk such as nutrient ingredients, amino acids and fatty acids were measured. The results showed that ingredients of yak milk, rate of colostrum milk protein and rate of milk fat were significantly higher (5.43 and 5.70 vs 4.84 and 4.57%, respectively) than that in the normal milk. Sixteen types of amino acids were detected in the yak milk with relatively higher content of glutamic acid, isoleucine, tyrosine and arginine and relatively lower content of lysine, glycine, methionine and phenylalanine. Total amino acid content (5.485%) in the yak colostrum was significantly higher than that in the normal milk (3.065%). Twenty-eight types of fatty acids, 19 types of saturated fatty acids and 9 types of unsaturated fatty were detected in the yak colostrum and mature milks. Myristic acid, 15-methyl palmitic acid, cis-octadecanoic acid, nonadecanoic acid, oleic acid and cis-11-oleic acid were the main fatty acids in the colostrum; While oleic acid, 15-methyl palmitic acid, stearic acid, 15-methyl hexadecanoic acid, nonadecanoic acid, heneicosanoic acid and behenic acid were the main fatty acids in the mature milk. Contents of oleic acid and stearic acid were significantly higher in the mature milk than in the colostrum. Results of this study could not only provide the basic data and reference material for understanding the nutritional value of yak milk but also help to promote the development and utilization of yak milk.

Key words: Yak milk, rate of milk protein, rate of milk fat, amino acid, fatty acid

INTRODUCTION

Yak (*Bos grunniens*) is a unique and valuable livestock resource that originated in the Qinghai-Tibetan Plateau. It is also regarded as famous cattle of the landscape. The characteristic features of the yak include its adaptability to cold, high altitudes, hypoxia, lack of grass and other harsh natural conditions of alpine. These characteristics led to its entry into the basic pastoral production, providing meat, milk, wool, cow down, leather, draft power, fuel, etc. Yak has irreplaceable ecological, pastoral, social and economic status in the alpines (Guo *et al*., 2012). Yaks live at an altitude of ≥3000 m. As there is no industrial pollution in alpine pastures, the yaks are rarely feed, totally depending on natural grassland for their food intake to maintain nutritional needs. Obviously, their milk is a type of nonpolluting green food with great nutritional values. The yak milk has many notable features: it is thick with pure incense, highly nutritious, rich in proteins and minerals and high rates of milk fat. However, due to the constraints of geographical conditions, production levels and other factors, the development and utilization of yak milk are still in very preliminary stage.

Qinghai Plateau yak is 1 of 13 local Chinese yak varieties. As yaks graze in natural and clean pastures, the yak milk is pure, natural and pollution-free with great qualities. Yak milk is easy to digest and absorb and hence provide an important source of food for the alpine herdsmen. In the alpine pastures, common dairy products mainly include fresh milk, butter, casein, cheese, yogurt, milk, cottage cheese, milk skin, etc. However, the rate of production of yak milk is low as the lactation period generally lasts for 150 days in adult yaks (weighing 220~350 kg). The mean total milk production is 487 kg and mean daily milk yield is 3.2 kg. To maximize the yak milk production and increase the added value of the yak milk products, a better understanding of the yak milk composition from Qinghai Plateau in different periods during lactation is required. This may provide basic data and scientific basis for the improvement of yak milk production.

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MATERIALS AND METHODS

Test animals: Yak distributed in the Qinghai-Tibet Plateau at an altitude of 3200 m above mean sea level with natural grazing conditions were included in this study.

Sampling method: Yak milk (>150 mL per yak) was randomly collected at the time of lactation from different yaks that graze naturally. Colostrum milk was collected within 3 days after delivery (April, hay stage) and mature milk was collected from lactating yaks during June (growth period of pasture) and September (lush period). Samples for analyzing milk composition and measuring amino acid were preserved at -20°C in refrigerator and samples for fatty acid composition were frozen at -78°C in the refrigerator.

Determination of milk composition: After taking the samples maintained at -20°C from the refrigerator, the samples were naturally thawed by placing them at a constant temperature (40°C) water bath (preheated homogeneous). Milk composition was measured using the multidairy analyzer (MilkScan FT120; Foss Analytical Denmark) including milk protein, milk fat, total solids, nonfat milk solids, lactose, density, freezing point and acidity.

Determination of the amino acid content in milk: After taking the samples maintained at -20°C from the refrigerator, the samples were naturally thawed by placing them at a constant temperature (40°C) water bath (preheated homogeneous). Amino acid contents in the Yak milk were determined using the automatic amino acid analyzer (Biochrom 30+; British Biochrom).

Determination of the fatty acid content of milk: Preparation of sample: methyl ester of the fatty acids of the milk was subjected to the following steps, as per the method described by Palmquist and Jenkina (2003). The samples were thawed in cold water after being refrigerated at -80°C and mixed well.

Milk sample (10 mL) was transferred to a 250 mL flask; approximately 100 mg coke gallic acid and undecanoic acid triglyceride internal standard solution (5.0 mg mL^{-1}) at 2 mL was added, a few grains of zeolite were added then 2 mL of 95% ethanol and 4 mL of ultrapure water were added and mixed well. Later, 5 mL ammonia (25%) was added and mixed well. The flask was placed in a water bath kept at 70-80°C for hydrolysis for 20 min. The flask was shaken at intervals of 5 min, so that the adhering particles were mixed into the flask solution. After the completion of hydrolysis, the flask was cooled to the room temperature and finally, 10 mL of 95% ethanol was added and mixed well.

After hydrolyzing the fats, the solution in the flask was transferred to a separatory funnel, the flask was rinsed using 50 mL of diethyl ether and the flask stopper was rinsed using a mixture of petroleum and ether (volume 1:1), the flush solutions were incorporated into the separatory funnel, stamped and shaken for 5 min and then allowed to stand for 10 min. The ether layer was collected in a 250 mL flask. This procedure was repeated thrice to extract the hydrolysate and finally the separatory funnel was rinsed with a mixture of diethyl ether and petroleum ether and collected in the 250 mL flask with volatile extraction solvent, the residue was the fat extract.

Saponification of fats and methyl ester of fatty acid: 8 mL of 2% sodium hydroxide solution was added to the 250 mL flask with fat extract and was collected with the reflux condenser and refluxed at 80°C water bath until the oil droplets disappeared. Methanol solution (7 mL) of 15% boron trifluoride was added from the upper end of the reflux condenser and refluxed at 80°C water bath for 2 min. The reflux condenser was rinsed with deionized water. The heating was stopped and the flask was removed from the water bath, quickly cooled to the room temperature, 10~30 mL of n-heptane (high-performance liquid chromatography grade) was accurately added and the flask was shaken for 2 min. Later saturated sodium chloride solution was added, about 5 mL of upper n-heptyl alkyl extraction solution was drawn and transferred to a 30 mL small test tube. About 5 g of anhydrous sodium sulfate was added and the flask was shaken for 1 min, left standing for 5 min and the top solution was drawn into the vial for determining the sample.

Chromatographic conditions: capillary column (cross-linked bonded stationary phase, containing 50% cyanopropyl) (60 m×0.25 mm, 0.25 μm). The injector temperature was 270°C and the detector temperature was 280°C. Temperature program: initial temperature was 130°C, duration of 1 min; 130~170°C, heating rate of 6.5°C min^{-1}; 170~215°C, heating rate of 2.75°C min^{-1}; held at 215°C for 12 min; 215~230°C heating rate of 4°C min^{-1}; held at 230°C for 3 min. Carrier gas: nitrogen; split ratio of 50:1; injection volume of 1.0 μL. Determination: 1.0 μL fatty acid methyl ester standard solution and each individual fatty acid methyl ester standard solution were injected into a gas chromatography-mass spectrometry (Agilent 6890N GC-5973 MSD; Agilent). The standard solution was measured in response to the value of chromatographic conditions (peak height or peak area)

and relative retention time of each fatty acid methyl ester (methyl carbonate relative XI) and their gas chromatographs were obtained. The response factor was calculated. Sample (1.0 μL) was injected into a gas chromatography-mass spectrometry; a sample response (peak height or peak area) was measured under the same conditions. After comparing the spectral response of the standard solution, the content of the fatty acid methyl ester in the sample was calculated.

Calculation of results: by comparing the relative peak retention times of the unknown samples and the known fatty acid methyl ester mixture standard samples, different types of fatty acids were identified and the relative amounts of the fatty acids were calculated in the chromatogram workstation with area normalization method using an integration process.

Data analysis: Three samples per group were determined. The same sample was measured twice and averaged. Analysis of variance was performed using SPSS 19.0. Results were expressed as mean±standard deviation. Least significant difference method was used for multiple comparisons and statistically significant difference was achieved if $p<0.05$.

RESULTS AND DISCUSSION

Nutrition analysis of yak milk: Nutritional contents (milk proteins, milk fats, total solids, nonfat solids, lactose, density, freezing point and acidity) of both colostrum and mature milk collected in different months from the yaks of Qinghai Plateau are shown in Table 1.

Amino acids: Amino acid content in the yak colostrum and mature milk was rich. Sixteen types of amino acids were detected (Table 2).

Fatty acid: Results of fatty acid contents of the yak colostrum and mature milk are shown in Table 3.

Composition of milk: Factors that influence the changes in the composition of milk include difference in species, interindividual differences, parity, stages of lactation, feeding and management levels, compositions of diet, modes of milking, seasons, temperature and state of health. Lawless *et al.* (1999) showed that the rate of milk fat and rate of milk protein of early and late lactation stages were significantly higher than that of mid-lactation stages. In this study, rate of milk protein and rate of milk fat and rate of colostrum milk were higher than other milks. At the same time, its nutritional content of dry matter, milk fat, lactose, minerals and other contents were also higher than other local milk products. Nutrient contents of yak milk changed with stages of lactation and seasons. But, the composition of lactose, density, freezing point, acidity and other contents were not significantly different in the same breed of yak colostrum and mature milks. Physical and chemical properties of the yak milk are not only the basic parameters for the development of dairy processing but also the necessary indicators to assess the nutritional value of milk from different animals.

Amino acid: Amino acids are the basic components of proteins. Different components of amino acids lead to specifically different molecular structures and biological

Table 1: Comparison of the nutritional contents of colostrum and mature milks of yak from Qinghai Plateau

Source	Protein (%)	Milk fat (%)	TS (%)	SNF	Lactose (%)	Density (g cm^{-3})	FPD (°C)	Acidity (°F)
Colostrum (April)	5.43±0.53^a	5.70±0.44^a	17.30±1.00^a	11.62±0.45^a	4.87±0.13^a	1038.0±2.0^a	0.668±0.005^a	12.39±1.44^a
Mature milk (June)	4.84±0.24^b	4.57±0.48^b	15.53±1.44	10.93±0.09^b	4.83±0.72^a	1035.5±1.7^a	0.679±0.014^a	11.96±1.25^a
Mature milk (September)	4.79±0.38^b	4.30±0.33^b	14.99±2.17^b	10.89±0.08^b	4.94±0.26^a	1036.6±2.4^a	0.669±0.04^a	11.32±1.65^a

Table 2: Amino acid content in the yak milk at different stages of lactation

Amino acid	Abbreviation	Colostrum (%)	Mature milk (June) (%)	Mature milk (September) (%)
Aspartate	Asp	0.420±0.010^a	0.250±0.014^b	0.230±0.014^b
Threonine	Thr	0.230±0.005^a	0.135±0.007^b	0.120±0.014^b
Serine	Ser	0.295±0.007^a	0.160±0.014^b	0.155±0.021^b
Glutamate	Glu	1.095±0.049^a	0.610±0.099^b	0.525±0.092^b
Glycine	Gly	0.115±0.007^a	0.070±0.003^a	0.070±0.004^a
Alanine	Ala	0.114±0.007^a	0.065±0.007^b	0.065±0.007^b
Valine	Val	0.335±0.008^a	0.180±0.141^b	0.185±0.007^b
Methionine	Met	0.130±0.004^a	0.070±0.002^b	0.065±0.007^b
Leucine	Leu	0.285±0.007^a	0.155±0.007^b	0.155±0.007^b
Isoleucine	Ile	0.540±0.028^a	0.315±0.007^b	0.300±0.014^b
Phenylalanine	Phe	0.265±0.007^a	0.135±0.007^b	0.155±0.007^b
Histidine	His	0.275±0.007^a	0.145±0.007^b	0.160±0.002^b
Lysine	Lys	0.130±0.004^a	0.070±0.003^a	0.065±0.007^a
Aspartate	Arg	0.505±0.007^a	0.290±0.014^b	0.275±0.007^b
Threonine	Pro	0.195±0.007^a	0.105±0.007^a	0.110±0.004^a
Serine	Tyr	0.555±0.049^a	0.310±0.014^a	0.310±0.007^a
Total Amino acid	TAA	5.485±0.049^a	3.065±0.219^b	2.945±0.177^b

The same superscript letters in the same column indicate no significant difference; different superscript letters in the same column indicate significant differences

Table 3: Results of Fatty Acid Analysis of yak Milk from Qinghai Plateau

No. fatty acid	Colostrum (April) (%)	Mature milk (June) (%)	Mature milk (September) (%)
Azelaic acid	0.497±0.054^{a}	0.580±0.048^{a}	0.139±0.093^{b}
Decanoate	0.373±0.081^{a}	0.107±0.013^{b}	0.544±0.018^{a}
Lauric acid (C12: 0)	0.047±0.021^{a}	3.117±0.501^{b}	1.483±0.166^{c}
10-Methyl-undecanoic acid	0.363±0.042^{a}	0.290±0.027^{a}	0.178±0.012^{b}
Tridecylic acid (C13: 0)	0.317±0.081^{a}	0.073±0.041^{b}	0.085±0.053^{b}
Myristic acid (C14: 0)	5.033±0.426^{a}	0.917±0.129^{b}	0.864±0.145^{b}
12-Methyl myristate	0.443±0.076^{a}	0.487±0.109^{a}	1.357±0.309^{b}
Pentadecylic acid (C15: 0)	0.363±0.051^{a}	0.193±0.022^{b}	0.069±0.038^{c}
14-Methyl-pentadecanoate	0.383±0.027^{a}	0.450±0.054^{a}	0.210±0.012^{a}
Palmitic acid (C16: 0)	0.097±0.010^{a}	0.537±0.059^{b}	1.240±0.070^{c}
Soft fat oleic acid (palmitoleic acid)	0.610±0.046^{a}	1.460±1.518^{b}	0.779±0.045^{a}
15-Methyl palmitate	27.460±2.723^{a}	21.137±1.816^{a}	6.701±1.367^{b}
Heptadecylic acid (C17: 0)	1.827±0.127^{a}	5.193±0.403^{b}	1.070±0.070^{a}
Oleic acid	10.777±1.765^{c}	14.240±1.349^{b}	27.157±3.034^{c}
Stearic acid (C18: 0)	0.660±0.071^{c}	6.463±1.404^{b}	8.543±1.288^{b}
Cis-11-octadecenoate	4.557±0.414^{a}	6.990±1.507^{a}	0.413±0.037^{b}
Cis-octadecanoic acid	20.49±1.742^{a}	0.350±0.008^{b}	0.328±0.017^{b}
Linoleic acid	0.233±0.034^{a}	1.117±0.164^{b}	0.447±0.021^{a}
8, 11-Octadecadienoic acid	3.813±0.286^{a}	3.237±0.368^{a}	0.280±0.026^{b}
6,9-Octadecadienoic acid	1.327±0.089^{a}	0.520±1.817^{b}	0.503±0.057^{b}
Nonadecanoic acid (C19: 0)	10.353±1.943^{a}	15.590±2.432^{a}	27.803±8.256^{b}
11, 14, 17-cis-eicosatrienoic acid	1.863±0.165^{a}	1.170±0.172^{b}	1.254±0.095^{b}
Heneicosanoic acid (C21: 0)	3.803±0.650^{a}	4.050±0.712^{a}	5.473±0.931^{b}
Cis-13-docosenoic acid	0.843±0.132^{a}	1.167±0.104^{a}	2.525±0.353^{b}
Docosahexaenoic acid	0.330±0.026^{a}	6.180±0.509^{b}	0.469±0.066^{a}
Behenic acid (C22: 0)	2.967±0.486^{a}	3.393±0.636^{a}	8.524±0.559^{b}
Lignoceric acid	0.150±0.011^{a}	0.880±0.052^{b}	0.317±0.033^{a}
28 acid (C28: 0)	0.040±0.006^{a}	0.120±0.026^{b}	1.493±0.635^{c}

The same superscript letters in the same column indicate no significant difference; different superscript letters in the same column indicate significant differences

activities of protein. Amino acids can be synthesized *in vivo* to tissue proteins; they can turn into acids, hormones, antibodies, creatine and other ammonia-containing materials or turn into carbohydrates and fats or oxidized to carbon dioxide, water and urea to produce energy. Therefore, the presence of amino acid in animals not only provides an important raw material for the synthesis of proteins but, also provides the material basis for the promotion of growth and normal metabolism to sustain life. Cow's milk can provide essential amino acids for human needs such as lysine, tryptophan, phenylalanine, methionine, threonine, isoleucine, leucine and valine. In this study, the total amino acid content was 5.485±0.0493 and 3.065± 0.219% in the yak colostrum (April) and mature milk (June), respectively. Contents of glutamic acid, isoleucine, tyrosine ammonia acid and arginine were relatively high and the contents of lysine, glycine, methionine and phenylalanine were relatively low with significantly higher rate of amino acid content in the colostrum than in the normal milk. Amino acid content (2.69%) of the Qinghai Plateau yak milk was 0.38% higher than that of the local Holstein milk. Meanwhile, the results showed that the amino acid content at different stages of lactation (June and September) did not differ significantly from each other indicating that there were no differences in amino acid contents of the mature milk in different seasons, which assured good quality.

Fatty acids: With research on milk fat, nutrition and health effects of fatty acids in milk and dairy products, their influence on the quality and flavor of dairy milk began to receive widespread attention. Recent studies have shown that milk fatty acids have specific health effects, especially conjugated linoleic acid which lowers the cholesterol, strengthens the immune system and inhibits the fat deposition and has anticancer, antidiabetic and other functions (Belury, 2002). Short- and medium-chain fatty acids, especially the water-soluble (e.g., C4:0, C6:0 and C8:0 and water-soluble and C12:0 is non-water-soluble) volatile fatty acids are important factors that affect the flavor of the product and lead to digestion. In this study, 28 types of fatty acids were detected in the Qinghai Plateau yak colostrum and mature milk, of which 19 types were saturated fatty acids and 9 were unsaturated fatty acids. The components in the colostrum were mainly myristic acid (C14:0), 15-methyl palmitic acid, cis-octadecanoic acid, nonadecanoic acid, oleic acid and cis-11-octadecenoate and the main components of the mature milk were 15-methyl palmitic acid, oleic acid, stearic acid, 15-methyl hexadecanoic acid, nonadecanoic acid, heneicosanoic acid and behenic acid. The saturated fatty acid content was 75.65% of the total fatty acids in the colostrum and the unsaturated fatty acid content was 24.35% of the total fatty acids. In the mature milk collected at June or September, the saturated fatty acid contents were 63.92 and 66.17% of the total fatty

acids, respectively and the unsaturated fatty acid contents were 36.08 and 33.83% of the total fatty acids, respectively. Oleic acid and stearic acid levels were significantly higher in the mature milk than in the colostrum.

With regard to saturated fatty acids, yak milk and other local milk products have similar characteristics. Long-chain fatty acid C14:0, C16:0 and C18:0 accounted for more than half of the total saturated fatty acids. The digestibility and absorbance properties of the long-chain fatty acids are not as good as that of medium-chain saturated fatty acids and unsaturated fatty acids. This is mainly due to the formation of complex long-chain saturated fatty acids in the small intestine and also the long-chain saturated fatty acids have high melting points and may have influence on the absorption of metal ions (such as calcium) or other fatty acids (Innis *et al*., 1994). Monounsaturated fatty acids reduce the incidence of cardiovascular diseases; oleic acid (C18: 1) can reduce the melting point of triglycerides, improve the liquidity and ability to metabolize fat globules and hence has a positive effect on the absorption of other fatty acids. Yak milk has relatively high oleic acid content. Polyunsaturated fatty acids have a variety of biological functions such as constitution of cell membrane, induction of gene expression and promotion of growth and development and prevention or treatment of cardiovascular diseases. Yak milk is rich in polyunsaturated fatty acids of linoleic acid, 8,11-octadecadienoic acid, 6, 9-octadecadienoic acid, 11,14,17-cis-twenty carbon leukotriene acid and docosahexaenoic acid. Understanding the fatty acid composition of the yak milk will help to promote the development and utilization of yak milk and increase the value addition of milk products.

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