Genetic Polymorphism of Milk Protein and Their Relationships with Milking Traits in Chinese Yak*

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ABSTRACT : Milk protein polymorphisms were genotyped by polyacrylamide gel electrophoresis (PAGE) from 109 Maiwa and 100 Jiulong yaks. The relationships between milk protein polymorphisms and 3 milking traits were studied. The results showed that β-CN, κ-CN and α-La were monomorphic, and α_{s1}-CN and β-Lg were polymorphic, with α_{s1}-CN D and β-Lg E as dominant genes, respectively. The frequencies of α_{s1}-CN D were 0.8073 and 0.6000 in two populations and β-Lg E were 0.9770 and 0.9700. The mean heterozygosities were 0.1021 and 0.1867 in the two populations. No significant effects on milking traits and milk protein compositions were observed except for α_{s1}-CN locus on fat percentage in Jiulong yak. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 11 : 1479-1483)***

Key Words : Milk Protein, Genetic Polymorphisms, PAGE, Milking Traits, Yak

INTRODUCTION

Milk protein secreted by the mammary epithelial cells contains mainly casein and whey milk protein. The genotypes of milk protein are controlled by co-dominant genes and conform to the Mendel's Laws. Milk protein types have received considerable research interests since the discovery of two variants of β-lactoglobulin in cow's milk by Aschaffenburg and Drewry (1955). The study of milk protein polymorphism is focused on three areas (Zhu et al., 2000). Firstly, milk protein polymorphism serves as an important component of genetic diversity, and is helpful in the conservation, exploitation and utilization of animal breeds. Second, the kinship of different animal breeds (or types) can be estimated and the origin and differentiation of animal breeds can be determined using the cluster figure developed from the gene frequency of polymorphic milk protein. Finally, to select excellent breeding animals, the quality of animal production and milking traits could be improved according to the linkage relationships between milk protein polymorphism and milking traits.

Maiwa and Jiulong yaks are two important breeds in China that belong to the bovine family, and they are the representative breeds of Qinhai-Tibet Plateau and Henduan Alpine type respectively (Cai, 1995). At present, there are many studies of genetic and phenotypic evaluation of milking traits, and/or their associations with milk protein polymorphisms for ordinary dairy cattle and their crossbreeds (Ostersen et al., 1997; Sharma et al., 2002; Singh et al., 2003), but there are few studies of genetic polymorphism of milk protein in yak (Kawamoto et al., 1992; Zhang et al., 2000,2002; Jiang et al., 2004). There appear to be no studies examining associations between genetic polymorphisms of milk protein and milking traits in yaks. The aim of this study was to study the genetic polymorphisms of milk protein and their relationships with milking traits and thereby to examine the genetic diversity as well as the genetic differentiation of yaks, and to provide a scientific basis for kinship of yak breeds (or groups) and marker assisted selection (MAS).

MATERIALS AND METHODS

Samples collection

Total of 109 mid-lactating period Maiywa, 100 Jiulong yaks and 15 Tibet yellow cattle were collected randomly from Sichuan Longri Breeding Farm and Jiulong country respectively. Forty ml mixed milk was sampled every morning during the study and brought to the laboratory after freezing. Ten ml milk had been taken out for electrophoresis analysis, the residue was prepared for the analysis of milk components. At same time, fifty ml milk of 20 Chinese Holstein were sampled from Chengdu Supo Dairy Cattle Farm for comparison.

Sample preparation

Ten ml milk was centrifuged at 1,000 rpm for 20 min at 4°C, underlayer skim milk were pipetted. 100 μl were taken to EP pipe for SDS-PAGE. The casein was separated from whey proteins by isoelectric precipitation at pH 4.6 with 1 mol/L HCl. After centrifugation at 1,000 rpm for 20 min at 4°C, the whey supernatant was stored at -20°C for electrophoresis. The casein pellet was washed twice with

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Table 1. The gene and genotype frequency of milk protein loci and genetic variation analysis of population in Maiwa yak and Jiulong yak

Loci	Genotype	Genotype frequency		Alleles	Gene frequency	
		Maiwa yak	Jiulong yak		Maiwa yak	Jiulong yak
α_{s1}-CN	BD	0.0092 (1)	0.0300 (3)	B	0.0138	0.0150
	BE	0.0183 (2)		C	0.1238	0.1200
	CC	0.1009 (11)	0.0600 (6)	D	0.8073	0.6000
	CD		0.0500 (5)	E	0.0551	0.2650
	CE	0.0459 (5)	0.0700 (7)			
	DD	0.7798 (85)	0.3800 (38)			
	DE	0.0459 (5)	0.3600 (36)			
	EE		0.0500 (5)			
β-CN	AA	1.0000 (109)	1.0000 (100)	A	1.0000	1.0000
κ-CN	BB	1.0000 (109)	1.0000 (100)	B	1.0000	1.0000
α-La	BB	1.0000 (109)	1.0000 (100)	B	1.0000	1.0000
	BB		0.0100 (1)	B	0.0230	0.0300
β-Lg	BE	0.0460 (5)	0.0400 (4)	E	0.9770	0.9700
	EE	0.9540 (104)	0.9500 (95)			
Mean homozygosity		0.8979	0.8133			
Mean heterozygosity		0.1021	0.1867			
Mean number of effective alleles		1.0000	1.2300			

acetate buffer (pH 4.6) and stored lyophilized at -20°C.

Milk yield test and analysis of nutritional components in milk

Daily milk yield was recorded on the spot and the milk yield in six months was estimated by the method of milk yield coefficient (Cai, 1995). The fat, protein and lactose in the milk were tested by automatic milking instrument (Milkscan-1340A/B, Denmark).

Electrophoresis of milk proteins

The electrophoresis and typing of casein and whey protein was done as proposed by Medrano and Sharrow (1989), contrasting by the milk of Chinese Holstein and Tibet yellow cattle. Alkaline gel electrophoresis to type α-, β- and κ-caseins was carried out using nondissociating discontinuous buffer system (pH 9.5). A stacking gel (0.375 M Tris-Hcl pH 6.7,4 M urea, 4.6% polyacrylamide, 0.1% TEMED and 0.63% APS) and a running gel (0.375 M Tris-HCl pH 8.9, 4 M urea, 8% polyacrylamide, 0.055% TEMED, and 0.062% APS) were utilized. The electrode chamber buffer was 0.025 M Tris-base, 0.2 M glycine, pH 8.3. Milk whey proteins were typed in a nondissociating, continuous buffer system. Polyacrylamide (14.2%) gels (0.375 M Tris-HCl pH 8.9, 0.055% TEMED, 0.062% APS) were run using the same pH 8.3 Tris-glycine electrode chamber buffer as outlined for the alkaline casein gels. Analysis of milk protein components in skim milk was determined by the method proposed by Laemmli (1970). The relative percentages of milk protein bands were quantified by light density instrument (CDS-200, Beckman, USA). The gel was stained by silver solution to observe the milk protein components (Merril et al., 1981).

Statistics

Gene and genotype frequency of milk protein loci was computed by the gene counting method (Chang, 1995). Mean homozygosity, mean heterozygosity, and mean number of effective alleles were calculated by the equation proposed by Nei (1983). Influences of milk protein loci on milking traits and milk components were analyzed by linear model without interaction as follows:

$$Y_{ijkl} = \mu + P_i + \alpha_{s1}\text{-}CN_j + \beta\text{-}Lg_k + e_{ijkl}$$

Where Y_{ijkl}=the observed value of milk yield, milk components or milk protein components; μ was population mean; P_i was fixed effect of parity; α_{s1}-CN_j was the fixed effect of α_{s1}-CN genotype; β-Lg_k was the fixed effect of β-Lg genotype; e_{ijkl} was random residual effect. All data were input by Excel and analyzed by software package SPSS (version 10.0).

RESULTS

The gene and genotype frequency of milk protein loci and genetic variation analysis of population in yaks

Table 1 shows the gene and genotype frequency of milk protein loci and genetic variation of population in yaks. Whereas β-CN, κ-CN and α-La were monomorphic, α_{s1}-CN and β-Lg were polymorphic in two breeds. The frequency of α_{s1}-CN DD was 0.7798 in Maiwa yak, the frequency of α_{s1}-CN DD and DE was 0.3800 and 0.3600 respectively in Jiulong yak. β-Lg EE was the dominant genotype in two breeds. The genotype distributions of α_{s1}-CN in two breeds and β-Lg in Jiulong yak deviate from the

Table 2. The least square mean (LSM) and standard error (SE) of the milking traits in Maiwa and Jiulong yaks

Breed or group	Sample size	Corrected milk yield for 153 days LSM±SE	Fat (%) LSM±SE	Protein (%) LSM±SE	Lactose (%) LSM±SE
Maiwa yak (full lactating)	89	228.62±8.48	5.12±0.13	5.09±0.56	5.00±0.05
Maiwa yak (half lactating)	20	190.10±10.56	6.24±0.23	6.02±0.54	4.73±0.04
Jiulong yak (full lactating)	87	266.74±9.40	6.25±0.50	4.95±0.98	4.91±0.11
Jiulong yak (half lactating)	13	176.89±13.25	7.41±0.48	6.41±0.50	4.66±0.17
Chinese holstein	20	6,508.50±10.65 (Corrected milk yield for 305 days)	3.14±0.34	3.04±0.65	4.77±0.25

Table 3. The least square mean (LSM) and standard error (SE) of the milk protein compositions and relative percentage in Maiwa and Jiulong yaks

Breed or group	Sample size	α-La (%) LSM±SE	β-Lg (%) LSM±SE	CN (%) LSM±SE	IgG-H (%) LSM±SE	BSA (%) LSM±SE
Maiwa yak (full lactating)	89	4.578±0.16	15.96±0.26	65.67±0.35	1.28±0.05	2.29±0.08
Maiwa yak (half lactating)	20	4.50±0.35	15.35±0.59	66.90±0.59	1.34±0.12	2.17±0.20
Jiulong yak (full lactating)	87	3.82±0.11	15.34±0.30	68.90±0.44	1.07±0.06	2.19±0.09
Jiulong yak (half lactating)	13	3.43±0.22	15.61±0.45	70.31±1.13	1.10±0.16	2.23±0.33
Chinese holstein	20	5.61±0.17	11.74±0.51	68.19±0.30	1.47±0.49	2.07±0.87

Table 4. The least square mean (LSM) and standard error (SE) of the milk fat percentage for different genotypes of α_{s1}-CN in Jiulong yak*

Genotypes	Sample size	Fat %	
		LSM	SE
BD	3	6.999^{abcd}	1.068
CC	6	5.979^{abcdef}	0.942
CD	5	7.567^{abcde}	0.828
CE	7	6.939^{abcd}	0.942
DD	38	6.048^{bcef}	0.648
DE	36	6.823^{abcde}	0.643
EE	5	4.977^{ef}	0.828

* Different superscripts in the same line differ significantly ($p<0.05$).

Hardy-Weinberg equilibrium, while β-Lg in Maiwa yak conformed to Hardy-Weinberg equilibrium. The mean homozygosity, mean heterozygosity and mean number of effective alleles of population were 0.8979, 0.1021 and 1.1100 in Maiwa yak and 0.8133, 0.1868 and 1.2300 in Jiulong yak.

Milk yield and nutrition component in milk

Table 2 shows the milk yield and nutritional components in milk of yaks and Chinese Holsteins. The fat percentage in Jiulong yak (full lactating and half lactating) were higher than in Maiwa yak ($p<0.05$). The fat and protein percentage in half lactating yak were higher than in full lactating yak in the two breeds ($p<0.05$). There were no significant differences in lactose percentage between yaks and Chinese Holsteins ($p>0.05$). The milk yield in half lactating yak were 83.15% and 66.32% of full lactating yak in two breeds respectively.

Relative percentage of milk protein composition in yak

There were six bands (α-La, β-Lg, α_{s1}-CN, β-CN, IgG-H and BSA) for SDS-PAGE of skim milk stained by Coomasie brilliant R250. Table 3 shows the relative percentage of milk protein composition scanning by light density instrument. The relative percentage of α-La and IgG-H in Maiwa yak was higher than in Jiulong yak, the relative percentage of CN in Jiulong yak was higher than in Maiwa yak. The relative percentage of β-Lg in yak was higher than in Chinese Holstein, but α-La was lower than in Chinese Holstein. There were no significant differences for relative percentage of BSA between yak and Chinese Holstein ($p>0.05$).

The effects of milk protein loci on milking traits and relative percentage of milk protein compositions

Table 4 shows the effects of milk protein loci on milking traits and relative percentage of milk protein composition. No significant effects on milking traits and milk protein composition were observed except for α_{s1}-CN locus on fat percentage in Jiulong yak. α_{s1}-CN CD was higher than DD and EE for fat percentage in Jiulong yak.

DISCUSSION

The polymorphism of milk protein in Tatong yak in Qinhai province showed that β-CN was monomorphic (β-CN AA), the genotype of α-La was BB expect one AB (1/82). It is similar to this research. There were three genes (B, C and E) for α_{s1}-CN, the dominant gene was α_{s1}-CN C. The β-Lg was monomorphic (β-Lg DD) (Zhang et al., 2000). The differences with this research arose from the different judging standard on milk protein polymorphism of yak.

The mean heterozygosity of populations were 0.1021 and 0.1867 respectively in Maiwa yak and Jiulong yak from five milk protein loci. This shows that the degree of genetic

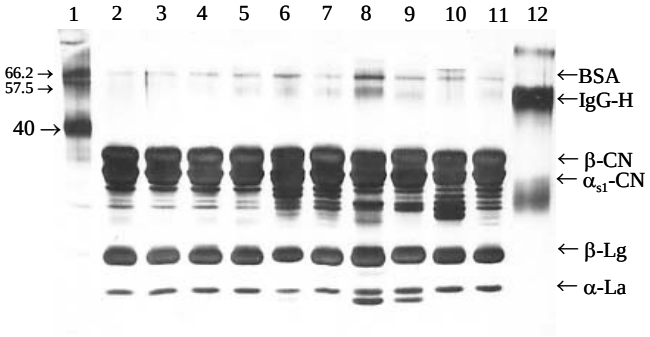

Figure 1. SDS-PAGE of skim milk from yak (silver stained). 1: height range molecular weight marker (40, 57.5, 66.2, 97.4, 116.2, 212 KD), 2-11: skim milk of yak, 12: purified yak IgG.

variation in Jiulong yak was greater than in Maiwa yak and provides clear evidence for the classification of two type breeds in China. But this is inconsistent with the study on MUC-I locus by Zheng et al. (2002) and historical data records. The mean heterozygosity of population from MUC-I locus was 0.673 and 0.537 in Maiwa and Jiulong yaks respectively and thereby genetic variation in Jiulong yak was greater than in Maiwa yak from locus MUC-I . A big decrease on quantity so called bottleneck effect in Jiulong yak was recorded by historical data record (Cai, 1995) and there was a habit of exporting yak and never importing yak in the producing area. Further more, there were relatively smaller numbers of Jiulong yak (about 50 thousand) compared with Maiwa yak (500 thousand) at the present time. The possible causes were the following: i) small sample sizes. ii) different breeding level for two yak populations. Jiulong yaks were raised by herdsmen scattered in different area in Jiulong county and natural (or random) mating is normal. But Maiwa yaks are different and controlled-mating was adopted to improve the milk traits in recent years. So the differences of genetic variation on milk protein polymorphism are mainly caused by different breeding system in two yak populations.

A great number of studies have shown that cow milk of the type κ-CN BB has a higher fat percentage, shorter coagulation time and higher curd firmness compared with κ-CN AA milk (Aleandri K et al., 1990; Jakob et al., 1992; Ostersen S et al., 1997), and the type β-Lg BB have higher fat percentage and protein percentage compared with β-Lg AB and β-Lg AA milk (Aleandri K et al., 1990). The yaks have high fat percentage and appropriate coagulation properties of milk because of the high gene frequency of κ-CN B (Zheng et al., 2001). According to the comparison of coagulation properties of yak milk and Chinese Holsteins, yak milk has a shorter coagulation time (8.08±0.22 min) compared with Chinese Holstein milk (12.02±0.51 min), and all of the yak milk samples coagulated normally after adding chymosin, while in Chinese Holsteins 35% of the milk samples failed to coagulate (Zheng et al., 2001). But the influences on fat percentage and protein percentage were undiscovered for β-Lg locus, the possible causes were the following. i) small sample size. ii) the natural condition of yak living were very poor. Yaks were living in the Qinhai-Tibet Plateau of altitudes above 3,000 metres. The annual average temperature was very low and there was no absolute non-frost period in any part of year. So it is very important to pay particular attention to the genetic foundation but also to improve the ecological environment and feeding conditions for the displaying the genetic potential and productive ability of yaks in China.

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