Asian-Aust. J. Anim. Sci.
Vol. 20, No. 2 : 166 - 171
February 2007

www.ajas.info

Association between PCR-RFLP Polymorphisms of Five Gene Loci and Milk Traits in Chinese Holstein

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ABSTRACT : The objective of this study was to assess the association of polymorphisms in *κ-cn*, β-*lg*, β-*lg* 5′ flanking region, *CSN1S2*, and *IGFBP*-3 genes with milk production traits and mastitis-related traits in Chinese Holstein. Traits analyzed were 305 day standard milk yield, protein percentage, fat percentage, the ratio of fat percentage and protein percentage, pre-somatic cell count, somatic cell count, and somatic cell score, respectively. *CSN1S2* locus was uninformative because only one genotype BB was found in Chinese Holstein. Allele frequencies of A and B in *IGFBP*-3 gene were 0.5738 and 0.4262 in Chinese Holstein population, which was different from reported Qinchuan cattle population. The genotypes of animals at *IGFBP*-3 locus significantly affected 305 day standard milk yield, protein percentage, and somatic cell score. The β-*lg* genotypes had a significant effect on protein percentage and the ratio of fat percentage and protein percentage. Polymorphism in β-*lg* 5′ flanking region was associated with 305 day standard milk yield, protein percentage, fat percentage, pre-somatic cell count, and somatic cell count. No significant associations of the polymorphism in κ-*cn* gene were observed for any trait. (**Key Words :** Chinese Holstein, β-*lg*, κ-*cn*, *IGFBP*-3, Milk Traits)

INTRODUCTION

Milk production traits (milk, fat, and protein yields; fat content; and protein content) are important quantitative traits, which have tremendous influence on dairy industry. Somatic cell score (SCS) has high genetic correlation with mastitis (0.60-0.80), and is one of the useful measures of mastitis at present (Banos and Shook, 1990; Chu and Shi, 2001). Although having made some progress using traditional selection methods for these traits, it is a money and time-consuming process. Whereas, marker assisted selection has greatly improved the accuracy and the intensity of these traits with high speed and low-cost. In dairy cattle breeding special attention is given to the genetic variation of candidate genes and its association with milk production traits and mastitis-related traits (Liefers et al., 2002; Kuss et al., 2003; Yahyaoui et al., 2003; Liefers et al., 2005; He et al., 2006; Taylor et al., 2006; Zhou et al., 2006).

Casein and whey protein are two main kinds of proteins, accounting for 95% of protein in milk. In fact, the polymorphisms of proteins are produced due to substitution, insertion or deletion of one or several bases of gene directing the synthesis of the milk proteins (Lin et al., 1999). Extensive studies examining the milk protein loci in cow have concluded that the alleles of these loci may be used as genetic markers of quantitative trait loci (QTL) for milk yield, composition and quality (Baxter, 1993; Ojala et al., 1997; Lin et al., 1999). Up to now, the results of surveys about allelic frequencies of milk protein genes and relationship between milk protein genotypes and milk production traits were not all the same. Additionally, there are differences between cattle breeds (Ojala et al., 1997).

Insulin-like growth factor binding proteins (IGFBPs) 3 is the major one of IGFBPs which play essential roles in prolonging half life and regulate biological activities of Insulin-like growth factors (IGFs), accounting for the binding of 90% of the plasma IGFs in a trimeric 150-kDa complex containing IGF, IGFBP-3, and an acid labile subunit (Baxter, 1993). IGFs can stimulate animal growth and mammary ductal development (Richert and Wood, 1999; Ge et al., 2001). So IGFBP-3 has the most important regulational function for IGFs biological activities and indirectly affects mammary gland development, growth and

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Received March 21, 2006; Accepted August 22, 2006

Table 1. Primer sequences of five loci and annealing conditions of polymerase chain reaction

Loci	Primer sequences	Annealing conditions	Sources
κ-*cn*	F:5′-cgctgtgagaaagatgaaagattc-3′	60°C 1 min	Zhao et al. (1999)
	R:5′-caacaattggtatactccttgaatct-3′		
β-*lg*	F:5′-cgctccccaccccgtcctcacc-3′	71.5°C 1 min	Zhao et al. (1999)
	R:5′-cccgtccccagtcaccccacagg-3′		
β-*lg* 5′ flanking region	F:5′-gtcactttccgtcctgggg-3′	63°C 1 min	Yahyaoui et al. (2000)
	R:5′-ggcctttcatggtctgggtga-3′		
CSN1S2	F:5′-tctcttgccatcaaaaca-3′	53.9°C 45 s	Ramunno et al. (2001)
	R:5′-tggtctttattcctctct-3′		
IGFBP-3	F:5′-ccaagcgtgagacagaatac-3′	60°C 1 min	Sun et al. (2003)
	R:5′-aggagggataggagcaagtt-3′		Macilla et al. (1997)

development traits, and meat quality of animals. Sun et al. (2003) showed that different genotypes of *IGFBP-3* affected several slaughter and carcass traits of Qinchuan cattle in China.

In this paper, PCR-RFLP was used to detect the polymorphisms in κ-*cn*, β-*lg*, β-*lg* 5′ flanking region, *CSN1S2*, *IGFBP*-3 gene, and the relationship between these genes and several milk traits and mastitis-related traits of Chinese Holstein were analyzed.

MATERIALS AND METHODS

Animal and data source

One hundred and sixty one without genetic relationships Chinese Holstein were used in this study. Traits analyzed were milk yield, protein percentage, fat percentage, somatic cell count (SCC), somatic cell score (SCS) and pre-somatic cell counts (PreSCC). Milk yield was measured twice a month, at the same time milk components were also measured. The total milk yield and average milk components of individuals were corrected. The total milk yield of individuals was adjusted to 305 days standard milk yield (Qiu, 1995).

DNA preparation, primer selection and genotyping

Genomic DNA of 161 Chinese Holstein was isolated from 2% heparin-treated blood samples and stored at -80°C, following standard procedures (Sambrook et al., 2002). The primer sequences and their annealing conditions were given in Table 1. The fragments of the five genes were amplified by standard PCR technology (Sambrook et al., 2002) with some modifications (Table 1).

Genotyping was carried out according to literatures. In brief, the PCR-RFLPs of these gene loci are bi-allelic. The PCR-RFLP-*Hw*26I of *CSN1S2* locus showed either undigested PCR product (310 bp, allele A), or 179 bp and 131 bp digestion products (allele B) as described by Ramunno et al. (2001). The PCR-RFLP-*Hin*dIII of κ-*cn* fragment showed either undigested PCR product (780 bp, allele A), or 413 bp and 367 bp digestion products (allele B) according to Zhao et al. (1999). Digestion of PCR fragment (651 bp) of *IGFBP*-3 gene with *Hae*III resulted in fragment lengths of 199, 164 , 154, 56, 36, 18, 16, 8 bp for genotype AA and 215, 164, 154, 56, 36, 18, 8 bp for genotype BB as described by Sun et al. (2003) and Macilla et al. (1997). After digestion with *Hae*III, the PCR product (434 bp) of β-*lg* gene showed either 300, 113, 21 bp digestion products (allele A), or 226, 113, 74, 21 bp digestion products (allele B) according to Zhao et al. (1999). Digestion of PCR fragment (710 bp) of β-*lg* 5′ flanking region with *Sma*I resulted in fragment lengths of 472, 181, 50, 7 bp for genotype AA and 472, 231, 7 bp for genotype BB as described by Yahyaoui et al. (2000).

Statistical methods

PIC (polymorphism information content), *h* (heterozygosity), *Ne* (effective number of alleles), and *S* (Shannon's information index) were calculated according to Bostein et al. (1980), Nei (1978), Kimura et al. (1973), and Guo (2002), respectively.

$$PIC = 1 - \sum_{i=1}^{m} p_i^2 - \sum_{i=1}^{m-1} \sum_{j=i-1}^{m} 2 p_i^2 p_j^2 \qquad h = 1 - \sum_{i=1}^{m} p_i^2$$

$$N_e = 1 / \sum_{ij}^{m} p_{ij}^2 \qquad S = -\ln \sum_{i=1}^{m} p_i \ln p_i$$

Where P_i, P_j are the allelic frequencies of the allele i and j. m is the total number of alleles.

The association between genotype and milk production traits was performed with the least square method (GLM procedure, SAS version 8.2, 2001). The model used to analyze the data was assumed to be:

$$Y_{ijkl} = \mu + G_i + S_j + L_k + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the observation of the trait, μ is the least square mean, G_i is the effect of marker genotype, S_j is the effect of season, L_k is the effect of lactation number and ε_{ijkl} is the residual effect.

Table 2. Allele and genotype frequencies at five loci in Chinese Holstein

Loci	Genotype frequencies			Allele frequencies	
	AA	*AB*	*BB*	p	q
κ-*cn*	0.6721	0.3279	0.0000	0.8360	0.1640
β-lg	0.1311	0.2787	0.5902	0.2705	0.7295
β-lg 5′ flanking region	0.3339	0.4879	0.1782	0.5778	0.4222
IGFBP-3	0.3607	0.4262	0.2131	0.5738	0.4262
CSN1S2	0.0000	0.0000	1.0000	0.0000	1.0000

Table 3. Genetic index in Chinese Holstein population

Loci	*h*	*Ne*	*PIC*	*S*
κ-*cn*	0.2742	1.3778	0.2366	0.4462
β -*lg*	0.3947	1.6521	0.3168	0.5838
β-*lg* 5′ flanking region	0.4879	1.9527	0.3689	0.6810
IGFBP-3	0.4891	1.9573	0.4439	0.6822
CSN1S2	0.0000	1.0000	0.0000	0.0000

RESULTS

Allele and genotype frequencies

Allele and genotype frequencies for the five loci were studied in Chinese Holstein (Table 2). Some genotypes were found at low frequency in Chinese Holstein. For example, no animals were homozygous BB at κ-*cn* locus. The *CSN1S2* locus was uninformative in this population. The allele B of *CSN1S2* locus seemed to be fixed in Chinese Holstein, or at a high frequency.

Genetic variation

Genetic index evaluated in Chinese Holstein population, heterozygosities (*h*), effective number of alleles (*Ne*), polymorphism information contents (*PIC*), and Shannon's information index were present in Table 3. Genetic index showed the genetic polymorphisms of these loci from high to low were *IGFBP-3*, β-*lg* 5′ flanking region, β-*lg*, κ-*cn*, and *CSN1S2*, respectively. *IGFBP-3*, β-*lg* 5′ flanking region, β-*lg* were moderate polymorphic loci in Chinese Holstein according to the criterion of *PIC* (Vaiman et al., 1994).

Relationship between the four loci genotypes and milk production traits as well as mastitis-related traits

Least squares means, and standard errors were shown in Table 4 for the effects of *IGFBP*-3, β-*lg*, β-*lg* 5′ flanking region, and κ-*cn* on milk production traits as well as mastitis-related traits. The polymorphisms at the *IGFBP*-3 gene were associated with 305 days standard milk yield, protein percentage, and SCS ($p<0.05$). For protein percentage and SCS, heterozygous animals were significantly higher than homozygous genotype BB. But animals with genotype AA and AB had lower 305 days standard milk yield than homozygous genotype BB ($p<0.05$).

β-*lg* had a significant effect on protein percentage and the ratio of fat percentage and protein percentage ($p<0.05$). Homozygous animals have less protein percentage than heterozygous genotype. For ratio of fat percentage and protein percentage, animals with allele B were lower than those with only allele A. While polymorphism in β-*lg* 5′ flanking region was associated with 305 days standard milk yield, protein percentage, fat percentage, pre-somatic cell count (PreSCC), and SCC ($p<0.05$; Table 3). Genotype AA had positive effects on 305 days standard milk yield and fat percentage while genotype BB on protein percentage and SCC. For PreSCC, heterozygous animals were much less than either homozygous genotype.

Table 4. Least squares means, and standard errors for the effect of *IGFBP*-3, β-*lg*, β-*lg* 5′ flanking region, and κ-*cn* on milk production traits as well as mastitis-related traits

Loci	Genotypes	305 yield	P%	F%	F/P	PreSCC	SCC	SCS
		LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE
IGFBP-3	AA	6,535.39^{a}±344.70	3.13^{ab}±0.04	3.45±0.07	1.18±0.03	740.13±109.90	921.57±139.23	4.09^{ab}±0.29
	AB	6,751.41^{a}±285.58	3.31^{b}±0.08	3.39±0.10	1.18±0.03	750.70±128.46	785.73±121.90	4.25^{b}±0.21
	BB	7,589.56^{b}±375.51	3.00^{a}±0.05	3.34±0.11	1.18±0.04	869.41±241.79	690.28±146.63	3.36^{a}±0.39
β-lg	AA	6,667.97±659.50	3.14^{b}±0.07	3.62±0.17	1.63^{b}±0.42	783.60±295.01	684.33±218.17	3.98±0.41
	AB	6,825.49±398.41	2.99^{a}±0.03	3.53±0.12	1.18^{a}±0.04	543.26±77.04	667.99±115.47	4.06±0.26
	BB	6,829.80±252.50	3.10^{b}±0.03	3.66±0.06	1.19^{a}±0.02	913.67±126.52	905.04±115.77	4.00±0.24
β-lg 5′ flanking region	AA	7,503.56^{b}±306.44	3.08^{a}±±0.04	3.79^{b}±0.09	1.24±0.03	889.00^{b}±141.90	890.66^{ab}±132.15	4.36±0.32
	AB	6,458.61^{a}±290.32	3.02^{a}±0.03	3.57^{ab}±0.08	1.32±0.13	498.76^{a}±68.58	524.85^{a}±74.21	3.94±0.18
	BB	6,934.37^{ab}±431.53	3.14^{b}±0.06	3.43^{a}±0.02	1.13±0.06	1092.20^{b}±250.97	1194.26^{b}±220.96	3.73±0.39
κ-*cn*	AA	6,785.36±214.02	3.07±0.03	3.63±0.07	1.27±0.09	799.48±112.09	840.62±99.88	4.13±0.02
	AB	7,056.13±450.86	3.06±0.04	3.53±0.09	1.15±0.03	669.03±97.25	778.96±126.79	3.87±0.03

305 yield, P%, F%, F/P, PreSCC, SCC and SCS represent 305 days standard milk yield, protein percentage, fat percentage, the ratio of fat percentage, protein percentage, pre-somatic cell count, somatic cell count, and somatic cell score respectively. LSM in a column with no common superscripts differ significantly ($p<0.05$).

No association was detected between κ-*cn* gene and any traits analyzed ($p>0.05$).

DISCUSSION

IGFBP-3 involved in the regulation of IGFs biological activities can promote the growth rate of animals (Mark et al., 2001). This study is the first known research using *IGFBP*-3 gene as a candidate gene for the milk traits. The genotype frequencies of *AA*, *AB*, *BB* on *IGFBP*-3 locus were 0.36, 0.42 and 0.22 respectively, the allele *A* and *B* frequencies were 0.57 and 0.43. While the genotype *BB* frequency on *IGFBP*-3 gene in Chinese Qinchuan cattle was only 0.02, the allele *A* and *B* frequencies were 0.84 and 0.16 (Sun et al., 2003). The heredity of *IGFBP*-3 gene, the different breeding aims and selective degree might be the main causes for the differences between Chinese Holstein and Qinchuan cattle. Association analysis showed that *IGFBP*-3 gene affects 305 days standard milk yield and protein percentage. The milk yield of genotype *BB* was significantly higher than that of genotype *AB* and AA. Thus allele *B* of *IGFBP*-3 gene might favor milk yield. The effect of *IGFBP*-3 gene on protein percentage exhibited that genotype *AB* is significantly higher than that of genotype *BB*, and the difference between genotype *AA* and *AB* was not significant. Association between milk protein gene and production traits has been extensively studied, but all results were not the same. Zhu and Zhang (2000) showed κ-*cn* locus and allele B of β-*lg* or its linked gene had a significant effect on protein percentage and fat percentage, whereas Lin et al. (1999) and Zhao et al. (1999) showed κ-*cn* locus was not associated with milk production traits. In this study, the result was consistent with Lin et al. (1999) and Zhao et al. (1999). In Zhao et al. (1999) study, milk component of genotypes at β-*lg* locus were significantly different, individuals with genotype AA had a lower protein percentage than that of either of genotypes. While in this study, genotype AB had a negative effect on milk percentage, which disagreed with Zhao et al. (1999).

Genetic variation in β-*lg* 5′ flanking region is one of the investigated hotspot. More attention are paid to the differentiated expression reason of β-*lg* A and β-*lg* B. Ford et al. (1993) found that the mRNA of β-*lg* A was much more than that of β-*lg* B, but the transcriptional level and stability were different. There are 14 nucleotides and 2 nucleotides replacement in 5′ flanking region and 5′ UTR of exon1 of β-*lg* A and β-*lg* B (Wagner et al., 1994). Ehrmann et al. (1997) found association between polymorphism of β-*lg* 5′ flanking region and protein percentage and β-*lg* volume in cow milk, and predicted that the difference was caused by effects of alleles on gene expression. Genetic variation of β-*lg* promotor region may explain the difference of milk production traits (Lum et al., 1997), and binding capacity of binding proteins led to the differentiated expression of alleles on relationship of SNP of AP-2 binding site with β-*lg* and milk yield (Kuss et al., 2003). Although reports have shown association between SSCP of β-*lg* control region, the difference could not attribute to special point mutation (Kaminski and Zabolewicz, 2000). So far, the molecular mechanism of effect of β-*lg* and its 5′ flanking region on milk production traits remains unknown.

In this study, we found a polymorphic locus in β-*lg* 5′ flanking region, and the genotype frequencies of β-*lg* 5′ flanking region in Chinese Holstein population agreed with Hardy-Weinberg equilibrium, which indicated the selection pressure on this locus was weak. The genotype of animals in β-*lg* 5′ flanking region was proved to have significant effects on milk yield, protein percentage, fat percentage, PreSCC, and SCC. More attention should be paid to relationship of genetic variation in β-*lg* 5′ flanking region and milk production traits in order to utilize them as effective markers in marker assisted selection.

Ramunno et al. (2001) found a new allele $CSN1S2^F$ at goat *CSN1S2* gene, which was caused by a nucleotide change (G→A, thirteenth nucleotide) in exon 3 eliminating restricted site of enzyme *Ahw*26I.

After digestion with *Ahw*26I, electrophoresis patterns showed allele $CSN1S2^F$ of *CSN1S2* locus was a fragment length of 310 bp, and the other allele (allele B) was two fragments length of 179 bp and 131 bp. In the present study, no carrier of $CSN1S2^F$ was detected in Chinese Holstein, which indicated that genetic characteristic of $CSN1S2^F$ was different between species.

Mean of genetic correlation of somatic cell counts and somatic cell scores with mastitis is 0.70, so SCC and SCS are two criteria to evaluate the health of udder and milk quality. Vast investigations focused on quantitative trait loci affecting SCC and SCS using microsatellite markers (Chu et al., 2003), several studies analyzed effects of candidate genes on SCC and SCS (Ryniewicz et al., 2002; Yahyaoui et al., 2000; Dietz et al., 1997; Starkenburg et al., 1997; Sharif et al., 1998). A polymorphic locus of defensin gene was tightly associated with SCC and milk production traits ($p<0.001$), which indicated this locus could be an effective marker to utilize in selection of udder health and milk production traits (Ryniewicz et al., 2002). In this study, we also analyzed associations between four polymorphic genes and mastitis-related traits. The results showed κ-*cn* and β-*lg* significantly affected milk production traits, but had no effect on SCC and SCS. While a significant effect was observed of genotypes of animals at *IGFBP-3* and β-*lg* 5′ flanking region on SCC and SCS, which may be caused by their functions of β-*lg* 5′ flanking region on milk protein gene expression and IGFs gene family in the process of animal development and growth.

ACKNOWLEDGMENTS

We wish to express our thanks to anonymous reviewers for the revision of our manuscript. This study was supported by grants from the Natural Science Foundation of P. R. China (No.30471238), the Higher Institute Natural Science Foundation of Jiangsu Province Educational Commission of P. R. China (No. 03KJD230216), Natural Science Foundation of Shaanxi Province of P. R. China (No.2004C122), and Graduate Student Education and Innovation Program of Northwest A&F University of P. R. China (No.05YCH018).

REFERENCES

Banos, G. and G. E. Shook. 1990. Genotype by environment interaction and genetic correlations among parities for somatic cell count and milk yield. J. Dairy Sci. 73:2563.

Baxter, R. C. 1993. Circulating binding proteins for the insulin growth factors. Trends Endocrin Met. 4:91-96.

Bostein, D., R. L. White, M. Sckolnick and R. W. Dvis. 1980. Construction of genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32:317-331.

Chu, M. X. and W. H. Shi. 2001. Brief discussion on mastits of dairy cattle. Chinese J. Dairy Cattle. 3:39-40.

Dietz, A. B., N. D. Cohen, L. Timms and M. E. Kehrli Jr. 1997. Bovine lymphocyte antigen class II alleles as risk factors for high somatic cell counts in milk of lactating dairy cows. J. Dairy Sci. 80:406-412.

Ehrmann, S., H. Bartenschlager and H. Goldermann. 1997a. Polymorphism in the 5' flanking region of the bovine Lactoglobulin encoding gene and its association with beta-lactoglobulin in the milk. J. Anim. Breed. Genet. 114:49-53.

Ford, C. A., M. B. Connett and R. J. Wilkins. 1993. β-lactoglobulin expression in bovine mammary tissue. Proc. New Zealand Soc. Anim. Prod. 53:167-169.

Ge, W., M. E. Davis, H. C. Hines, K. M. Irvin and R. C. Simmen. 2001. Association of a genetic marker with blood serum insulin-like growth factor-I concentration and growth traits in Angus cattle. J. Anim. Sci. 79:1757-1762.

Guo, M. C. 2005. Research on the in formatics model of population genetic variation. Dissertation for doctor degree of Northwest A & F University. China.

He, F., D. X. Sun, Y. Yu, Y. C. Wang, Y. Zhang. 2006. Ass ociation between SNPs within prolactin gene and milk perf ormance traits in Holstein dairy cattle. Asian-Aust. J. Anim. Sci. 19:1384-1389.

Kaminski, S. and T. Zabolewicz. 2000. Associatons between bovine beta-lactoglobulin polymorphism within coding and regulatory sequences and milk performance traits. J. Appl. Genet. 41:91-99.

Kimura, M. and T. Ohta. 1973. Mutation an evolution at the molecular level. Genetics. 73(Suppl.):19-35.

Kuss, A. W., J. Gogol and H. Geldermann. 2003. Association of a polymorphic AP-2 binding site in the 5' flanking region of the bovine β-lactoglobulingene with milk proteins. J. Dairy Sci. 86:2213-2218.

Liefers, S. C., M. F. W. te Pas, R. F. Veerkamp and T. van der Lende. 2002. Associations between leptin gene polymorphisms and production, live weight, energy balance, feed intake, and fertility in Holstein heifers. J. Dairy Sci. 85:1633-1638.

Liefers, S. C., R. F. Veerkamp, M. F. W. te Pas, Y. Chilliard and T. van der Lende. 2005. Genetics and physiology of leptin in periparturient dairy cows. Domest Anim. Endocrin. 29: 227-238.

Lin, F. Y., L. Li and Y. F. Chen. 1999. Analysis of the relationship between genotype of bovine κ-casein and milk yield. Chinese J. Anim. Sci. 35:8-9.

Lum, S. L., P. Dove and J. F. Medrano. 1997. Polymorphisms of bovine β-lactoglobulin promoter and differences in the binding affinity of activator proteion-2 transcription factor. J. Dairy Sci. 80:1389-1397.

Macilla, H. J., H. M. Zhang and S. K. Denise. 1997. A novel polymorphism in the bovine insulin-like growth hormone factor binding protein-3 (IGFBP-3) gene. Anim. Genet. 28:375-378.

Mark, H. V., A. I. Bettina and N. B. Benhard. 2001. IGF-I treatment reduces hyperphagia, obesity, and hypertension in metabolic disorders induced by fetal programming. Endocrin. 142:3964-3973.

Nei, M. 1978. Estimation of average hetorozygosity and genetic distance from a small number of individuals. Genet. 89:583-590.

Ojala, M., T. R. Famula and J. F. Medro. 1997. Effects of milk protein genotypes on the variation for milk production traits of Holstein and Jersey cows in California. J. Dairy Sci. 80:1776-1785.

Qiu, H. 1995. Cattle Production. Chinese Agriculture Press, Beijing, China.

Ramunno, L., G. Cosenza, M. Pappalardo, E. Longobardi, D. Gallo, N. Pastore, P. Di Gregorio and A. Rando. 2001. Characterization of two new alleles at the goat CSN1S2 locus. Anim. Genet. 32:264-268.

Richert, M. M. and T. L. Wood. 1999. The insulin-like growth factors and IGF type I receptor during postnatal growth of the muyine mammary gland: sites of mRNA expression and potential functions. Endocrin. 140:454-461.

Ryniewicz, Z., L. Zwierzchowski, E. Bagnicka, J. Krzyzewski and N. Strzalkowska. 2002. Preliminary investigations on the polymorphism of defensin genes in cattle-relation with milk somatic cell count. Anim. Sci. Papers and reports. 20:125-131.

Sambrook, J. and D. W. Russell. Translated by Huang Pei Tang. 2002. Molecular Cloning A Laboratory Manual. 3nd. Science Press, Beijing, China.

Sharif, S., B. A. Mallard, B. N. Wilkie, J. M. Sargeant, H. M. Scott, J. C. M. Dekkers and K. E. Leslie. 1998. Association of the bovine MHC DRB3 alleles with occurrence of disease and milk somatic cell score in Canadian dairy cattle. Anim. Genet. 29:185-193.

Starkenburg, R. J., L. B. Hansen, M. E. Kehrli Jr. and H. Chester-Jones. 1997. Frequencies and effects of alternative DRB3. 2 alleles of BoLA for Holsteins in milk selection and control lines. J. Dairy Sci. 80:3411-3419.

Sun, W. B., H. Chen, X. Q. Lei, C. Z. Lei, Y. H. Zhang, R. B. Li, L. S. Zan and S. R. Hu. 2003. Polymorphisms of insulin-like growth factor binding protein 3 gene and its associations with

several carcass traits in Qinchuan cattle. Hereditas (Beijing). 25:511-516.

Taylor, V. J., D. E. Beever, M. J. Bryant and D. C. Wathes. 2006. Pre-pubertal measurements of the somatotrophic axis as predictors of milk production in Holstein–Friesian dairy cows. Domest. Anim. Endocrin. 31:1-18.

Vaiman, D., D. Mercier, K. Moazami-Goudarzi, A. Eggen, R. Ciampolini, A. Lépingle, R. Velmala, J. Kaukinen, S. L. Varvio, P. Martin, H. Levéziel and G. Guérin. 1994. A set of 99 cattle microsatellites characterization synteny mapping and polymorphism. Mamm. Genome. 5:288-297.

Wagner, V. A., T. A. Schild and H. Geldermann. 1994. DNA variants within the 5'-flanking region of milk protein-encoding genes. II. The β-lactoglobulin-encoding gene. Theor. Appl. Genet. 89:121-126.

Yahyaoui, M. H., A. Angiolillo, F. Pilla, A. Sanchez and J. M. Folch. 2003. Characterization and genotyping of the Caprine κ-casein variants. J. Dairy Sci. 86:2715-2720.

Yahyaoui, M. H., R. N. Pena, A. Sanchez and J. M. Folch. 2000. Rapid communica tion: Polymorphism in the goat β-lactoglobulin proximal promotor region. J. Anim. Sci. 78:1100-1101.

Zhao, C. J., Y. Zhang and N. Li. 1999.Research on the relationship between molecular genetic polymorphism of milk protein and dairy traits in Chinese Holsteins. J. Yellow Cattle Sci. 25:13-15.

Zhou, L., Y. Y. Yang, Z. H. Li, L. J. Kong, G. D. Xing, H. S. Di and G. L. Wang. 2006. Detection and characterization of PCR-SSCP markers of the bovine lactoferrin gene for clinical mastitis. Asian-Aust. J. Anim. Sci. 19:1399-1403.

Zhu, M. X. and Y. Zhang. 2000. Analysis of correlation between milk protein polymorphisms and dairy performance of Chinese Holstein in Beijing. Chinese J. Anim. Sci. 36:3-6.