Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

# The Impact of Kappa Casein Gene Polymorphism on Milk Components and Other Productive Performance Traits of Chinese Holstein Cattle

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ARTICLE HISTORY

Received: October 24, 2010
Revised: November 01, 2010
Accepted: November 04, 2010

**Key words:**
Kappa casein genetic variance
Milk total solids and non solids
Parity
Season of calving

**ABSTRACT**

Polymorphism of kappa casein gene (κ-Cn) at exon 5 in 259 Chinese Holstein cattle was investigated using polymerase chain reaction-single strand conformation (PCR-SSCP) technique, to verify its effect on milk composition (fat, protein, lactose, total solids (TS) and solids not fat (SNF) content and influence of other factors such as parity and season of calving; aiming at utilizing it as a genetic aid in selection to improve the quality of production of this herd. A 218 bp fragment containing exon 1V of kappa casein gene was amplified by PCR and SSCP was applied to identify the structural gene polymorphism of κ-Cn. General linear model (GLM) was used to analyze differences between genotypes. The results indicated that κ-Cn genotypes significantly ($P<0.05$) affected fat, protein and lactose content. But it had no effect ($P>0.05$) on TS and SNF. However, cows with genotype TT showed higher fat and protein contents (3.89 and 3.50%, respectively) than those of genotypes CC and TC (3.62 and 3.66% for fat; 3.35 and 3.36 % for protein, respectively). Except for lactose content; parity had no effect on milk components. Also, the results disclosed that season of calving did not affect ($P>0.05$) milk composition. This study indicated that the κ-Cn genetic variants may be used as a genetic aid through increasing the frequency of desired genotypes to improve the quality of production of this herd.



**To Cite This Article:** Hamza AE, ZP Yang, XL Wang, RJ Chen, HT Wu and AI Ibrahim, 2011. The impact of kappa casein gene polymorphism on milk components and other productive performance traits of Chinese Holstein cattle. Pak Vet J, 31(2): 153-156.

## INTRODUCTION

Milk components in cattle are quantitative traits, being influenced by environmental and genetic factors (allelic variations at many loci). The bovine milk specific proteins include casein four genes vis: $\alpha_{s1}$-, β-, $\alpha_{s2}$- and κ-casein (*CSN1S1*, *CSN2*, *CSN1S2* and *CSN3*, respectively) producing approximately 80 percent of the protein content of cow's milk (Farrell *et al*., 2004). These proteins and their genetic variants have been widely investigated, and considered as an important factor associated with lactation performance, milk composition and cheese yield efficiency (Aleandri *et al*., 1990). The casein genes are tightly linked and inherited as a cluster so they have a potential value and can play an important role in marker-assisted selection for milk traits (Lien and Rogne, 1993). The κ-Cn gene has been intensively studied due to its vital role in the processing properties of milk by providing colloidal stability to the casein micelle. Its molecule is a single-chain polypeptide of 169 amino acids with a molecular weight of 19.2 KDa (Di Stasio and Mariani, 2000). The bovine κ-Cn gene possesses 5 exons distributed over approximately 13 kb of the bovine genome (Martin *et al*., 2002). Moreover, several single nucleotide polymorphisms have been detected in 5'-flanking region of the casein genes (Threadgill and Womack, 1990). This genetic polymorphism of milk proteins can be a helpful aid of selection and an informative marker in breeding research. Relationships between milk protein polymorphism, production traits, composition of milk and milk manufacturing properties have been investigated and described in several studies (Ng-Kwai-Hang, 1998). However; literature reported contradicting results on this relationship. While some studies (Ju *et al*., 2008) elucidated no significant associations; results from several other authors (Khatkar

*et al*., 2004; Rachagani and Gupta, 2008; Riaz *et al*., 2008; Ju *et al*., 2009) confirmed that there is a relationship. The aim of the present study was to investigate the effect of κ-Cn gene polymorphisms on milk components in Chinese Holstein cattle.

## MATERIALS AND METHODS

### Samples collection and DNA extraction

A total of 259 Chinese Holstein cattle blood samples were collected from the experimental farm of Yangzhou University in year 2009. The samples were taken from each cow from the jugular vein in a 10ml vacuum tube containing acid citrate dextrose and stored in deep freezer at −20°C pending to DNA extraction. Genomic DNA was extracted using proteinase K digestion followed by standard phenol-chloroform extraction protocol (Mullenbach *et al*., 1989). The quantity and quality of DNA were measured by spectrophotometer at 260/280nm using an Eppendorf BioPhotometer (Germany). The content of DNA was estimated by ultraviolet spectrophotometer (Germany), and the genomic DNA was diluted to 50ng/μL.

Data concerning milk performance (parity and season of calving) and milk composition (fat%, protein%, lactose%, and total solids TS) were obtained from Dairy Herd Improvement Association records. Solids not fat (SNF) was calculated by subtracting fat% from the milk total solids% (SNF = TS% - Fat %).

### PCR amplification

A 218 bp fragment containing exon 1V of kappa casein gene was amplified by PCR using forward 5′CTAAATCTGGCATAAAAGTA′3 and reverse 5′AATCACGGACTAAATAA′3, primers with accession No AY380228 sequence from gene bank. PCR was carried into 20μL final volume containing 100 ng template, 1μL 8 pmol/μL each primer, 0.4μL 10 mmol/μL dNTP, 1.0□2.4μL 25 mmol $MgCl_2$, 0.3μL 5 U Taq DNA polymerase and 2μL 10×buffer. PCR amplification reactions were used as follows: 94°C for 5min (initial denaturation), followed by 30 cycles of (denaturation) 94°C for 1min then (annealing) 50.6°C for 1min and (extension) at 72°C for 1min, and (final extension) at 72°C for 10 min. DNA implication was verified by electrophoresis of the PCR product with loading dye (95% formide, 0.25% bromophenol blue and 0.25% xylene cyanol) on 1.5% (W/V) agarose gel in 1X TAE, using DNA marker to confirm the desired PCR products length.

### Single strand conformation polymorphisms

Single strand conformation polymorphism (SSCP) analysis is said to be one of the most accurate and reliable technique for the identification of structural gene polymorphism that occurs as a result of point mutation (Neibergs *et al*., 1993). A total of 2.0μL PCR product was mixed with 8μL of the denaturation solution (50mmol/L NaOH, 1mmol/L EDTA), and 1μL of the loading buffer containing 0.25% bromophenol blue and 0.25% xylene cyannole, denatured for 10min at 98°C, and rapidly chilled at -20°C. The samples were then electrophoresed in 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A thermostatically controlled refrigerated circulator was used to maintain constant temperature (4°C) of the gels. The gels were run in the following conditions: 250V, 40mA, 10min and 150V, 24mA for 8h. The gel was then silver stained. The patterns of DNA bands were observed and photographed with the GDS7500 System (UVP). Amplified PCR products of the different bands were directly sequenced by Shanghai Sangon Biological Engineering Technology & Services CO, Ltd, Shanghai, China.

### Statistical analysis

Data pertinent to milk components of different genotypes were subjected to analysis of variance (ANOVA) using the general linear model (GLM) applying Statistical Analysis Software (SAS Institute Inc., 2000). The following statistical model was used:

$$Y_{ijkl} = \mu + G_i + A_j + S_k + e_{ijkl}$$

Where $Y_{ijkl:}$ is the observation on each trait of the *ijkl*th animal, $\mu$ is the general mean of each trait, $G_i$ is the fixed effect of *i*th kappa casein genotype, $A_j$ is the fixed effect of *j*th parity number, $S_k$ is the fixed effect of the *k*th sex and *eijkl* is the random error effect associated to the *ijkl*th observation.

## RESULTS AND DISCUSSION

Table 1 shows the effect of κ-Cn genotypes, parity and season of calving on milk composition in Chinese Holstein cattle. The results indicated that κ-Cn genotypes had significant ($P<0.05$) effect on fat, protein and lactose content, but it did not affect either total solids or solids not fat. It was observed that cows with genotype TT had significantly higher fat (3.89%) than those of genotypes CC and TC (3.62 and 3.66%, respectively); and also had insignificantly higher proteins (3.50%) than those of other two genotypes (3.35 and 3.36%, respectively),

These results coincide with those reported by various workers (Ng-Kwai-Hang *et al*., 1990; Alipanah *et al*., 2007; Sitkowska *et al*., 2008; Botaro *et al*., 2009; Nilsen *et al*., 2009). All these studies showed an individual effect of K-Cn gene on milk fat and protein content. However, our results are different from those reported by Ju *et al*. (2009) who studied polymorphisms of κ–casein gene at exon 4 and 5 and its association with milk performance traits in Chinese Holstein cattle and found that cows of TC and CC genotypes had significantly ($P<0.05$) higher protein content than those of genotype AA. On the other hand, our results disagree with literature data demonstrated by Strzalkowska *et al*. (2002), who claimed that K-Cn gene had no significant influence on milk components. Also, our results are not in line with those obtained by Lunden *et al*. (1997) and Tsiaras *et al*. (2005) who found that lactose yield and content were not significantly affected by κ-Cn genotype. These contradicting reports can be referred mostly to some factors such as population size, breed frequency of occurrence of specific variants under study, way of treating and demonstrating performance traits and the statistical approach applied.

The results shown in Table 1 elucidated that except for lactose content; parity number had no effect ($P>0.05$) on milk components (fat, protein, TS and SNF content).

**Table 1:** Effect of casein genotypes, parity number and season of calving on milk composition (%)

| Parameter | Fat | Protein | Lactose | TS | SNF |
|---|---|---|---|---|---|
| K-casein genotype | | | | | |
| CC | 3.62±0.72$^{b}$ | 3.35 ± 0.49$^{a}$ | 4.85 ± 0.33$^{ab}$ | 12.9 ± 1.06$^{b}$ | 9.36 ± 0.71$^{a}$ |
| TC | 3.66 ± 0.64$^{b}$ | 3.36± 0.45$^{a}$ | 4.87 ± 0.33$^{a}$ | 13.1 ± 1.05$^{ab}$ | 9.41± 0.60$^{a}$ |
| TT | 3.89 ± 0.77$^{a}$ | 3.50 ± 0.50$^{a}$ | 4.76 ± 0.40$^{b}$ | 13.3 ± 1.17$^{a}$ | 9.38 ± 0.66$^{a}$ |
| Parity | | | | | |
| 1 | 3.71± 0.57$^{a}$ | 3.40 ± 0.43$^{b}$ | 4.93 ± 0.29$^{a}$ | 13.3 ± 0.86$^{a}$ | 9.55 ± 0.48$^{a}$ |
| 2 | 3.71 ± 0.72$^{a}$ | 3.38± 0.40$^{b}$ | 4.95 ± 0.30$^{a}$ | 13.2± 0.94$^{a}$ | 9.43 ± 0.56$^{ab}$ |
| 3 | 3.73± 0.73$^{a}$ | 3.35 ± 0.54$^{b}$ | 4.82 ± 0.25$^{a}$ | 13.0 ± 1.16$^{a}$ | 9.30 ± 0.67$^{ab}$ |
| 4 | 3.72 ± 0.93$^{a}$ | 3.44 ± 0.48$^{ab}$ | 4.63 ± 0.35$^{b}$ | 12.9 ± 1.49$^{a}$ | 9.32 ± 0.83$^{ab}$ |
| 5 | 3.94 ± 0.67$^{a}$ | 3.63 ± 0.58$^{a}$ | 4.54 ± 0.58$^{b}$ | 13.2 ± 1.27$^{a}$ | 9.24 ± 0.82$^{b}$ |
| Season of calving | | | | | |
| Autumn | 3.68± 0.62$^{a}$ | 3.47 ± 0.49$^{a}$ | 4.87 ± 0.35$^{a}$ | 13.2 ± 0.97$^{a}$ | 9.44 ± 0.64$^{ab}$ |
| Spring | 3.94 ± 1.13$^{a}$ | 3.44 ± 0.55$^{a}$ | 4.88 ± 0.23$^{a}$ | 13.5± 1.65$^{a}$ | 9.69 ± 0.98$^{a}$ |
| Summer | 3.80 ± 0.92$^{a}$ | 3.40 ± 0.79$^{a}$ | 4.79± 0.36$^{a}$ | 13.1 ± 1.47$^{a}$ | 9.30 ± 0.79$^{b}$ |
| Winter | 3.74 ± 0.70$^{a}$ | 3.38 ± 0.49$^{a}$ | 4.80 ± 0.37$^{a}$ | 13.1± 1.06$^{a}$ | 9.33 ± 0.59$^{b}$ |
| Overall | 3.74 ± 0.72 | 3.41 ± 0.48 | 4.82 ± 0.33 | 13.1 ± 1.11 | 9.39 ± 0.65 |

Figures bearing different superscripts in a column for each parameter differ significantly (P<0.05).

Moreover, fat and protein contents tended to increase with increasing parity. In contrary, lactose, TS and SNF contents declined with increasing parity. Similar findings were reported by Tyrisevä (2008), who demonstrated that parity number did not influence both fat and protein contents; however, in the present study as these findings declined with increasing parity. Our results agree with those obtained by Kunaka and Makuza (2005) who found that protein content of Holstein-Frisian population of Zimbabwe increased with increasing parity up to parity 6 then a big increase to parity 7. On the other hand, the present results are not in line with those reported by Schultz *et al*. (1990) who demonstrated that fat and protein percentages decreased with increased parity.

Analysis of variance (Table 1) revealed that season of calving had no effect (P>0.05) on milk components. Similarly, Teixeira *et al*. (2003) found no effect of seasonality on fat and the other milk solids.

## Conclusion

Milk components in cattle (fat, protein, lactose, TS and SNF) are quantitative traits, being influenced by genetic and environmental factors. This study demonstrated that K-Cn genotypes had significant effect on fat, protein and lactose content, but it did not affect either TS or SNF. Moreover; the study observed that cows with genotype TT had higher fat and protein contents than those of genotypes CC and TC. Further researches with large numbers of animals are required to investigate these associations between K-Cn genotypes and milk components of Chinese Holstein cattle. This study also indicated that the κ-Cn genetic variants may be used as a genetic aid through increasing the frequency of desired genotypes to improve the quality of production of this herd.

## Acknowledgement

The research was financially supported by the National "863" Program of China (No. 2008AA10Z144) and the Natural Science Foundation Research of University of Jiangsu Province (09KJA230002 and 08KJB230005), China.

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