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Kappa Casein Gene Polymorphism and its Impact on Milk Yield and Reproductive Performance Traits of Chinese Holstein Cattle

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Abstract: The effect of Kappa Casein (K-CN) gene polymorphism on milk yield (305 day yield) and some reproductive performance (number of service/conception, calving interval, open period and gestation period) was investigated using 237 Chinese Holstein cattle. The polymorphism of K-CN was detected by Polymerase Chain Reaction-Single Strand Conformation (PCR-SSCP) technique. The obtained results elucidated that K-CN genotypes did not affect ($p>0.05$) 305 day milk yield; cows with genotype TT produced slightly higher milk than those of TC and CC genotypes. However, the effect of K-CN genotypes on reproductive performance revealed that K-CN genotypes were not significant ($p>0.05$) for any traits.

Key words: Holstein Chinese cattle, Kappa casein gene polymorphisms, PCR-SSCP, single nucleotide polymorphism, reproductive performance, China

INTRODUCTION

Bovine milk contains six major milk protein sub-groups known as (α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein, α-lactalbumin, β-lactoglobulin) which are controlled by codominant autosomal genes according to Mendelian inheritance exist in different allelic forms (Cardak, 2005).

In the past few decades, after accomplishment of amino acids sequences of Kappa Casein (K-CN) main variants (A and B) by Grosclaude *et al.* (1972) and Mercier *et al.* (1973) many studies (Jakob and Puhan, 1992; Pytlewski *et al.*, 2002; Ju *et al.*, 2009) have indicated that there are possible associations between certain genetic variants of milk protein and production performance traits, milk components and overall technological properties.

Despite of the huge studies on the association of milk protein polymorphism with productive traits, only few researches reports (Hargrove *et al.*, 1980; Jairam and Nair, 1983; Tsiaras *et al.*, 2005; Felenczak *et al.*, 2008) have been carried-out regarding their association with reproductive performance.

To get maximum use of milk protein genes polymorphisms as a selection criterion in cattle breeding programs to improve the quantity and quality of milk production performance traits; it is necessary to consider the reproductive traits. This triggered the interest to commence this study to investigate the effect of genetic polymorphism of Kappa Casein (K-CN) on milk yield and reproductive performance in Chinese Holstein Cattle.

MATERIALS AND METHODS

Samples collection and DNA extraction: A total of 237 Chinese Holstein Cattle blood samples were collected from the experimental farm of Yangzhou University. Samples were taken from each cow from the jugular vein in a 10 mL 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.

The quantity and quality of DNA were measured by spectrophotometer at 260.280 nm using an Eppendorf BioPhotometer (Germany).

The content of DNA was estimated by ultraviolet spectrophotometer (Germany) and the genomic DNA was diluted to 50 ng μL^{-1}.

Data concerning milk production: A 305 day yield and reproductive performance traits (number of service/conception, calving interval, open period and gestation period) were obtained from Dairy Herd Improvement association DHI records.

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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'AATC ACGGACTAAATAA'3, primers with accession No AY380228 sequence from gene bank. PCR was carried into 20 μL final volume containing 100 ng template, 1 μL 8 pmol μL^{-1} each primer, 0.4 μL 10 mmol μL^{-1} dNTP, 1.0-2.4 μL 25 mmol $MgCl_2$, 0.3 μL 5 U Taq DNA polymerase and 2 μL 10 × buffer.

PCR amplification reactions were used as follows: 94°C for 5 min (initial denaturation) followed by 30 cycles of (denaturation) 94°C for 1 min then (annealing) 50.6°C for 1 min and (extension) at 72°C for 1 min 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: A total of 2.0 μL PCR product was mixed with 8 μL of the denaturation solution (50 mmol L^{-1} NaOH, 1 mmol L^{-1} EDTA) and 1 μL of the loading buffer containing 0.25% bromophenol blue and 0.25% xylene cyannole, denatured for 10 min 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, 40 mA, 10 min and 150V, 24 mA, for 8 h. 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 and Services CO, Ltd, Shanghai, China.

Statistical analysis: The data on the reproductive traits and 305 day milk yield of the different genotypes were subjected to Analysis of Variance (ANOVA) using the General Linear Model (GLM) from the Statistical Analysis Software (SAS Institute Inc., 2000). The statistical model used was:

$$Y_{ijkl} = \mu + S_i + A_j + G_k + e_{ijkl}$$

Where:

Y_{ijkl} = The observation on each trait of the ijklth cow
μ = The general mean of each trait
S_i = The covariate effect of the season of calving
A_j = The covariate effect of parity number
G_k = The fixed effect of the kth genotype
e_{ijkl} = The random error effect associated to the ijklth observation

RESULTS AND DISCUSSION

Data pertinent to milk yield and reproductive performance of cows are shown in Table 1. The obtained results indicated that different K-CN genotypes had no significant ($p>0.05$) effect on 305 day milk yield. It was observed that cows of TT genotype produced 15.4 and 217.7 kg higher than both cows of TC and CC genotypes, respectively. These results were in consistent with those reported by Haenlein *et al.* (1987), Ng-Kwai-Hang *et al.* (1984, 1990) and Ju *et al.* (2008) all of them found no relationship between milk yield and K-CN variants. However, the present results were not in line with those stated by other researchers (Ng-Kwai-Hang *et al.*, 1986; Eenennaam and Medrano, 1991; Tsiaras *et al.*, 2005; Sitkowska *et al.*, 2008) who found that K-CN genotypes affected significantly milk yield.

Results concerning the effect of K-CN genotypes on reproductive performance revealed that K-CN genotypes were not significant ($p>0.05$) for any traits (Table 1). K-CN genotypes had no significant ($p>0.05$) effect on number of service/conception, however cows having CC-K-CN genotype needed fewer service per conception compared with the others having (TC and TT) genotypes. These results agree with findings of Ng-Kwai-Hang *et al.* (1990) and Tsiaras *et al.* (2005) but were not in line with those reported by Ronda and Perez-Beato (1983) who demonstrated that K-casein locus had a significant effect on service per conception.

Table 1: Effect of K-CN genotypes on reproductive traits and 305 day milk yield

Parameters	No.	No of services/ conception	Calving interval (days)	Open period (days)	Gestation (days)	No.	305 day milk yield (kg)
Season of calving	-	NS	NS	NS	*	-	NS
Parity no.	-	*	*	*	NS	-	*
Genotype	-	NS	NS	NS	NS	-	NS
CC	68	2.19^a±1.43	483.47^a±151.91	203.91^a±151.05	279.56^a±5.29	61	7341.5^a±1365.3
TC	86	2.35^a±1.49	454.19^a±131.03	175.21^a±130.30	278.98^a±7.16	76	7543.6^a±1518.7
TT	83	2.25^a±1.30	463.12^a±144.64	183.04^a±145.96	280.08^a±5.99	84	7559.0^a±1381.4

Means without a common superscript differ significantly ($p<0.05$)

The effect of K-CN genotype on calving interval was not significant ($p>0.05$), however cows of TC κ-CN still there is a paucity of studies investigating the association between genetic polymorphism of κ-CN and reproductive performance traits.

CONCLUSION

The results of the present study indicated that polymorphism of κ-CN had no significant effect on milk yield and reproductive performance traits of Chinese Holstein Cattle however cows having TC κ-CN genotype had shorter calving interval and less number of days from first service to conception than the TT and CC types. Further research is needed to clarify the association between K-CN polymorphism and reproductive performance traits.

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