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Effects of κ-Casein Variants on Milk Yield and Composition in Dairy Cattle

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Abstract

The effect of κ-casein (κ-CN) variant on milk production traits (milk yield, fat yield, protein yield, fat percentage and protein percentage) was estimated for 568 Holstein cows in the first lactation. The κ-CN variants were determined by PCR-RFLP (restriction fragment length polymorphism) technique at the DNA level. Single trait linear model was used for the statistical analysis of the data. Results of this study indicated that κ-CN variants affected significantly milk yield (P<0.05) and protein yield (P<0.01). Animals with the BB variant produced 622kg milk more and had protein yield higher by 32kg compared with animals with the AA variant. No associations between the κ-CN variants and other milk production traits were found. Therefore, milk and protein yield may be improved through milk protein typing by increasing the frequencies of κ-CN B variant in dairy cattle population. In cheese making, it will be also preferable to have milk with the B variant of κ-CN, which gives higher yield having a better quality than the A variant milk.

Key words : milk protein, κ-CN variant, DNA typing, milk production traits

Introduction

Milk proteins, synthesized and excreted by the mammary epithelial cells during lactation, provide the sulking calf with a source of minerals and amino acids. Six proteins, comprising 95% of the total protein in bovine milk, have previously been classified according to their biochemical characteristics into the four caseins, αs_1, αs_2, β and κ, and β-lactoglobulin (β-LG) and α-lactalbumin (α-LG) (Threadgill and Womack, 1990). Considerable phenotypic variation is found within milk proteins. These protein variants are detected because of substitutions and deletions in their amino acid sequences that are coded for by alleles milk protein genes (Eigel *et al.*, 1984). Milk proteins are of growing economic interest because of their direct relationship with milk quality, composition and cheese making properties (Aleandri *et al.*, 1990; Lodes *et al.*, 1996; Falaki *et al.*, 1997). Various studies suggest important relationships between milk protein variants and milk production traits and cheese manufacturing. Thus, there is great interest in using the milk protein variants as genetic marker for better milk quality, altering milk composition and improving the physicochemical properties of milk for the manufacture of various dairy products. In particular, rapid progress could be achieved by selecting sires containg the favorable milk protein variants. Milk protein variants have been classified by gel electrophoresis of milk samples. However, milk protein analyses are limited to mature lactating cows and genotyping sires demands the analysis of multiple dam/daughter pairs (Ng-Kwai-Hang *et al.*, 1991). Recently, developments in molecular genetics and gene analysis have made it possible detect these milk protein differences at the DNA level by examining genomic DNA extracted from blood, milk, hair or semen. The DNA-based typing permits the determination of an animal's variant regardless of age and sex (Cowan *et al.*, 1992).

The κ-casein (κ-CN) is a phosphoglycoprotein that constitutes approximately 12% of the casein complex of bovine milk. The amount and type of κ-CN present in milk vary considerably depending on the individual cow. κ-CN plays an important role in the formation, stabilization and aggregation of the casein micelles thus altering the manufacturing properties and digestibility of milk (Mercier *et al.*, 1973). Owing

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to these properties, the κ-CN variants might affect protein structure, which is strongly connected with production traits and biological fitness. The purpose of this study was to investigate possible associations between κ-CN variants and milk yield and compositions in the dairy cattle.

Materials and Methods

Animals and Data

DNA samples were collected from 568 Holstein cows in several commercial dairy farms registered in the official performance testing program. The following 305-d milk production traits of cows in first lactation records were used in the statistical analyses: milk yield, fat content (%), fat yield, protein content (%) and protein yield. Means and standard deviations of the milk production traits for the Holstein dairy cow population examined in this study are in Table 1.

DNA Extraction from Milk Samples

3mL of milk are diluted 1:1 in 50 mM Tris-HCl (pH 7.6) and centrifuged at 2,500rpm for 10 min. The cell pellet is resuspended in a washing solution(10 mM Tris-HCl, pH 7.5, 1mM EDTA, pH 8.0) and transferred to Eppendorf tubes. The cells are briefly centrifuged in microcentrifuge and the resulting cell pellet is resuspended in Lysis buffer-K (10 mM Tris, pH 8.3, 50mM KCl , 0.5 % Tween , 0.4% Proteinase K). The suspension is incubated at 56℃ for 45 min and subsequently at 95℃ for 10 min. After ultra centrifugation for 1 sec, the DNA samples were stored in the refrigerator.

PCR-RFLP Analysis

For analysis of the κ-CN variants, the DNA between nucleotide 10592 and 11466 of the κ-CN gene was amplified by the use of the oligonucleotide primer sequences described by Chung *et al.*(1995): 5'-gTgcCTgAgTAggTATCCTAg-3' and

Table 1. Means and standard deviation of 305-d milk production traits from 568 first lactation cows

Trait	$\overline{X}$	SD
Milk yield, kg	7,981	1,580
Fat content, %	3.72	0.42
Protein content, %	3.12	0.19
Fat yield, kg	298	43.3
Protein yield, kg	253	35.7

5'-gTAgAgTgCAACAACACTgg- 3'. The PCR reaction was performed in 100 μL volumes containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μL of each dNTP, 100 ng of genomic DNA, 100 pmol of each primers and 2.5 units of *Taq* DNA polymerase. Amplification was as follows: initial denaturation at 94℃ for 5 min, annealing at 57℃ for 1 min, extension at 74℃ for 3 min and final extension of 10 min in a DNA Thermal Cycler (Perkin-Elmer Cetus Corp). After DNA amplification, the PCR products were digested with *Hind*III and restriction fragments were then separated by electrophoresis in 1.5 % agarose gels. The gel was stained with ethidium bromide and visualized under UV light.

Statistical Analyses

The following linear model was used to test the effects of κ-CN variants on first parity production of milk, fat and protein and on fat and protein contents. The PROC GLM procedure of the SAS (SAS, Inst. Inc., Cary NC)) was used for statistical analyses.

$$Y_{ijkl} = \mu + H_i + (Y \times S)_j + \kappa\text{-}CN_k + e_{ijkl}$$

Y_{ijkl} = the production record of the l^{th} cow
μ = overall means
H_i = the effect of the i^{th} herd
$(Y \times S)_j$ = the effect of the j^{th} year and season of calving
$\kappa\text{-}CN_k$ = the effect of the k^{th} κ-CN variant (k=AA, AB or BB)
e_{ijkl} = the random residual associated with each record.

Results

We have amplified an 874 bp fragment between nucleotides 10592 and 11466 from exon IV to intron IV of the bovine κ-CN gene. Fig. 1 shows the restriction patterns of κ-CN variants after digestion with the *Hind*III enzyme. Three different κ-CN variants AA, AB and BB were observed, with frequencies of 0.536, 0.403 and 0.061, respectively (Fig. 2). Estimated allelic frequencies were 0.74 and 0.26 for A and B. The results from the analysis of effects of κ-CN variants on milk yield and composition are shown in Table 2. The κ-CN variant affected milk yield (P<0.05) and protein yield (P< 0.01). Cows with the BB variant produced higher milk yield than cows with AA variant (P<0.05). BB type cows produced 622 kg of milk more than AA type cows (Fig. 3). Also, cows with BB variant had higher protein yield compared with AA or AB

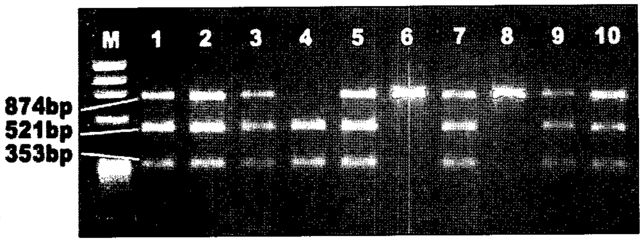

Fig. 1. PCR-RFLP patterns of κ-CN variants in 1.5% agarose gel following digestion with *Hind*III enzyme of PCR products. In the gel, lanes 6 and 8 represent AA type, lane 4 represents BB type and lanes 1, 2, 3, 5, 7, 9 and 10 represent AB type. M represents the molecular size marker(ΦX - 174 DNA/*Hae*III marker).

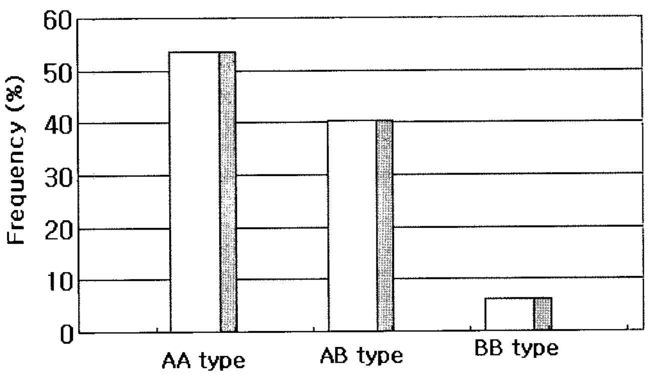

Fig. 2. Frequencies of k-CN variants AA, AB and BB in domestic Holstein cow population.

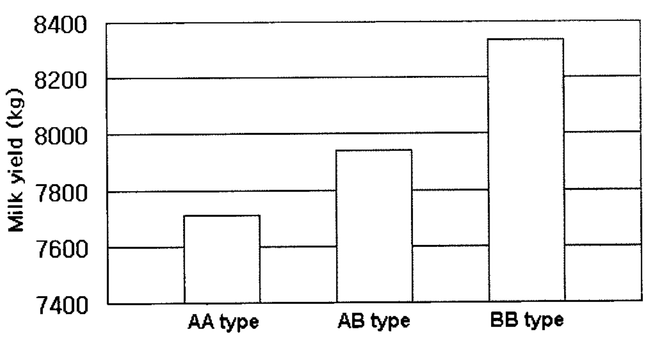

Fig. 3. Association between κ-CN variants and 305-d milk yield for first lactation in Holstein.

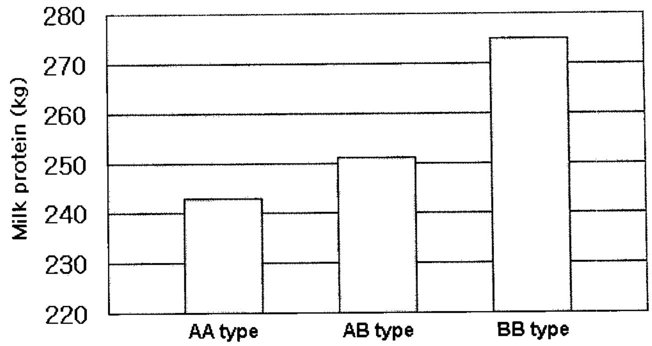

Fig. 4. Association between κ-CN variants and milk protein yield for first lactation in Holstein.

variant cows ($P<0.01$). BB type cows produced more protein by 32 and 24kg, respectively, compared with AA and AB type cows (Fig. 4). However, fat yield, fat content and protein content were not significantly affected by the κ-CN variant.

Discussion

The κ-CN gene has been extensively studied in cattle for its stabilizing role of the casein micelles and, therefore, its influence on the manufacturing properties of milk. To date, six variants have been described: A, B, C, and D and the recently discovered F and G (Barroso *et al.*, 1998). The κ-CN A and B are the most common variants in European cattle such as Holstein breed. κ-CN A variant differs from B by having Ile(ATC) and Ala(GCT) at amino acid positions 136 and 148 (Alexander *et al.*, 1988). The most prevalent variant was A and the B variant showed very low frequency. These frequencies were similar to those reported in the literature for Holstein populations (Ng-Kwai-Hang *et al.*, 1986; Aleandri *et al.*, 1990; Famula and Medrano, 1994). The effects of bovine κ-CN on milk yield, milk components and cheese manufac-

Table 2. Least square means and standard errors of κ-CN variant for the milk production traits

κ-CN variant	Milk production traits				
	Milk	Fat	Protein	Fat	Protein
	(kg)			(%)	
AA	7,713±268.3 [a]	295±16.82	243±20.45 [a]	3.77±0.17	3.11±0.18
AB	7,940±242.6 [a,b]	304±17.04	251±15.29 [a]	3.68±0.14	3.16±0.16
BB	8,335±223.5 [b]	308±15.18	275±12.36 [b]	3.72±0.13	3.18±0.12
P	*	-	**	-	-

* $P<0.05$, ** $P<0.01$.
[a,b] Means with different superscripts in the same column are significantly different.

turing have been extensively studied. However, published results on the effect of κ-CN variants on milk production traits have not always been consistent. It has generally been agreed that κ-CN B variant is associated with higher milk protein yield (Ng-Kwai-Hang *et al.*, 1984; Van Eenennaam and Medrano, 1991; Tsiaras *et al.*, 2005) and protein content (Gonyon *et al.*, 1987; Ng-Kwai-Hang *et al.*, 1990, Bovenhuis *et al.*, 1992; Van den Berg *et al.*, 1992). Increased milk yield associated with the B variant of κ-CN have been found in other studies (Ng-Kwai-Hang *et al.*, 1986; Lin *et al.*, 1989; Van Eenennaam and Medrano, 1991), whereas other studies have indicated no effect (Ng-Kwai-Hang *et al.*, 1984; Lin *et al.*, 1986 ; Ng-Kwai-Hang *et al.*, 1990; Lunden *et al.*, 1997). In this study, the B variant of κ-CN was also significantly favourable for milk and protein yields. In addition, the variants of milk protein may be of importance to the technological properties of milk. The chemical difference by the substitution of one or two charged for non-charged amino acids between the variants of the proteins affects various physicochemical properties of milk (Van den Berg *et al.*, 1992). The κ-CN B variant is related to an increased total protein content compared to the A variant (Aleandri *et al.*, 1990). This is mainly due to the increased casein content (Ng-Kwai- Hang *et al.*, 1986). Cheese yield is greatly influenced by the casein content of the milk. The bovine κ-CN BB variant has been associated with the production of milk superior manufacturing properties (Van Eenennaam and Medrano, 1991). The use of milk from cows typed κ- CN BB in cheese making results in a shorter rennet coagulation time, the formation of a firmer curd and production of a greeter cheese yield as compared with milk from κ-CN AA cows (McLean and Schaar, 1989). In conclusion, it may be desirable to select for cows containing κ-CN B variant for higher milk yield and milk protein, and improved cheese yield and quality in dairy industry.

Acknowledgements

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