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Sire Selection for Milk Production Traits with Special Emphasis on Kappa Casein (CSN3) Gene

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Abstract: One of the possibilities to progress in farm animal is selection of sire with favorable genotypes of genetic markers for milk production traits. Milk is an essential source of energy, proteins, minerals and vitamins for young in the first period of their life. The use of ruminant milk and milk products in human nutrition extends throughout life for a large majority of the population. Caseins comprise about 80% of the total protein content of milk and present polymorphism with changes in the amino acid sequence. Within this abundance of proteins, kappa-casein is noteworthy, since it has been associated with differences in milk yield, composition and processing. The objective of this review was to observe the existence of polymorphism in the kappa-casein gene for selecting the best alleles as an aid for genetic improvement of bulls.

Key words: Sire Selection · Milk Proteins · Kappa-casein gene · Genotyping

INTRODUCTION

The aim of the breeding industry is to identify genetically superior bulls and maximize the number of offspring conceived with their semen through artificial insemination (AI), thus increasing the dissemination of their genes. One way to achieve this objective is through the selection of sires that increase the genetic potential of the herd to produce more milk, milk fat, or milk protein [1].

For sire selection in cattle breeding, special attention was given to the genes of milk proteins [2]. *Gibson et al.* [3] suggested that milk protein loci could be used in the future routine procedures as additional selection criteria for dairy sires. Genotyping of milk proteins, such as k-casein gene is extremely important for selection practice to improve industrial milk production [4-6].

A variety of genetic variants of the caseins are found, suggesting that the caseins have evolved at an unusually high rate. This might be possible because their function does not depend on an exact protein structure [7]. Therefore, the casein genes not only show a high rate of point mutation but also a high rate of major re-arrangements like deletions and insertions.

Milk Proteins: Milk proteins synthesized and excreted by the mammary epithelial cells during lactation which support the suckling calf with a supply of minerals and amino acids [8]. The major milk proteins consist of the four caseins, αs1-casein (CSN1S1), β-casein (CSN2), αs2-casein (CSN1S2) and κ-casein (CSN3), as well as the two major whey proteins, α-lactalbumin (LALBA) and β-lactoglobulin (LGB) [9,10]). These previous six proteins represent 95% of the total proteins in bovine milk [11].

Studies in dairy cattle pointed out the association between milk protein genetic variants and milk production traits [12, 13]. In dairy sheep, similar studies are scarce and basically restricted to Mediterranean dairy breeds [14].

Milk Caseins: The polymorphism of the milk casein gene has been associated to differences in milk composition, processing and quality [15,16] and also with yield characteristics [17].

Caseins which composed of $\alpha_{s1,}$ $\alpha_{s2,}$ β and κ-casein represent a major part of bovine milk protein and due to their nutritional importance and commercial significance they are the most extensively studied milk proteins [18]. Caseins are found conjugated with colloidal calcium phosphate in particles known as casein micelles [7, 19]. Casein, with its content in milk being about 2.6% (26 g/kg milk) [20] and is the most important milk protein for the cheese industry [21]. The quantity of αs1-casein is 10 gm/kg milk, αs2-casein is 2.6 gm/kg milk, β-casein is 10.1 gm/ kg milk and κ-casein is 3.3 gm/ kg milk [20].

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Milk caseins compose the major protein component of ruminant milk and are secreted in the form of stable calcium phosphate micelles, so caseins elevate both calcium and phosphate concentrations in milk by forming stable micelles with calcium and phosphates. Moreover, the secretion of caseins in the micelle form allows maintaining low viscosity of the milk although the high protein concentration. These casein micelles are important in protein and mineral nutrition in offspring, also important in determining the physical properties of milk [22].

Naturally, casein should provide the calf with nutrients. But, not only amino acids are involved; Ca and phosphate are important components as well. Obviously, casein has evolved in such a way that it can bind large amounts of the poorly soluble calcium phosphate while keeping these substances in a stable suspension [20].

Casein genes have been studied intensively because of their highly regulated expression (tissue, stage, sex and age specific) and because, in domestic animals, they have a major impact on milk and cheese production [23]. Phosphorylation of caseins occurs in Golgi apparatus post-translational by the action of casein kinases [24].

Both structure and organization of the casein gene locus have been described in human, mouse and bovine [25, 26] and mapped on chromosome 6 in both bovine and caprine species [9, 27]. In bovine, Ferretti *et al.* [28], Threadgill and Womack [9], Lien and Ronge [29] and Mercier and vilotte [30] reported that, the casein genes locate within a 200 kb region on the chromosome six in the order α_{s1}-, α_{s2}-, β- and κ-casein.

Kappa Casein (κ-Casein): Although milk casein composition varies considerably between livestock species [31], κ-casein seems to be ubiquitous in accordance with its biological role [32]. The performance of technological processes of cheese production depends on the structure of Kappa casein gene [33]. Kappa casein is located primarily on the surface of the micelles, determining the size of the micelles and stabilizing their structure whereas the calcium-sensitive caseins are generally thought to be located predominantly within the micelles [34, 35].

Kappa-casein plays a very important role in stabilizing the casein micelles and curd formation as when cleaved at a specific phenylalanine bond by chymosin, causes initiation of micelle aggregation [36]. Kappa-casein is important for the renneting properties during curd formation. During this process, hydrolysis of κ-casein by chymosin (rennin), between amino acid No. 105 and 106, leads to destabilization of the micelles and the formation of a gel [37]. The gel may trap milk fat and whey proteins and is the first step in cheese production [38].

Kappa-casein protein is 169 amino acids long with varying regions at codons 136 and 148. The A variant has threonine (ACC) at codon 136 and aspartic acid (GAT) at codon 148, whereas the B variant has isoleucine (ATC) and alanine (GCT) at codons 136 and 148, respectively [39].

Lien and Ronge [29] and Leveziel *et al.* [40] stated that, the bovine κ-casein gene consists of a microsatellite repeat in intron 3 with six alleles specifically. The Kappa-casein gene bovine composed of 13 kb sequence divided into 5 exons. Characterization of k-casein gene was determined by Alexander *et al.* [41]. K-casein is located in a region 95-120 kb downstream of the αs1-gene in bovine genome [26].

Genetic Variants of Kappa Casein (CSN3): The Kappa-casein (CASK) 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, E [42] and the recently discovered F and G [43, 44]. Many studies reviewed by Grosclaude [45] demonstrate the influence of genetic variants of k-casein on the manufacturing properties of milk, especially those of importance in cheese technology. Milk containing the B variant shows better lactodynamographic properties than milk containing A [46], E [47], or C [48] variants.

In cattle, the kappa-casein gene (CSN3) presents two common genetic variants, A and B [49. The variant CSN3 B is associated with processing properties, such as cheese making [15, 50]. These alleles differ by substitutions in two amino acids, at positions 136 and 148 (alanine) [39]. In many countries, an increase in frequency and a superiority of allele CSN3 B was observed, when the progeny tested demonstrated a greater frequency of genotype CSN3 BB in cattle. The genotypes BB and AB are used in artificial insemination programs, to obtain a greater increase of the frequency of these alleles in commercially interesting cattle populations.

In buffalo bull, Abdel Dayem *et al*. [6] demonstrated that all studied animals were monomorphic for the kappa-casein gene. They possess only allele B in homozygosis form. Patel *et al*. [4] reported a polymorphism in buffalo kappa-casein gene but that the BB-genotype frequency (0.968) was very much higher than the AB genotype (0.032).

Some authors have identified CSN3 genotypes by RFLP using cDNA sequences [51, 52] and by PCR-RFLP in Bos taurus [53, 54] and in buffalo [56]. Both polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques were developed to distinguish κ-casein A, B alleles [57], κ-casein A, B, C, E, alleles [58], κ-casein F, G alleles [44] and κ-casein H allele [59] at the DNA level.

Mitra *et al.* [60] who used the PCR-RFLP technique with restriction enzymes Hind III, Hinf I and Taq I. This technique made it possible to detected polymorphism in alleles A and B of the kappa-casein gene in Sahiwal cattle (Bos indicus) and in Murrah, Nili-Ravi and Egyptian buffaloes (Bubalus bubalis). The PCR product produced a 379 bp DNA fragment. The enzymes Hind III and Taq I produced two fragments of allele B: 156 and 223 bp and 123 and 256 bp, respectively.

Milk containing the B allele of κ-casein has cheese making properties and a higher milk protein percentage than milk with the A allele [61]. However, CSN3 B has not been found to possess certain advantages in manufacturing properties, but it leads to increase in the cheese production by eight to ten percent [17].

According to many authors, κ-CN B would be more desirable than κ-CN A because it is associated with higher casein content in milk [62 - 66], higher cheese-yielding capacity [67,68], more favorable cheese composition [67, 69] and better coagulating properties in terms of rennet clotting time and curd firmness [62, 70-72]. So, it seems preferable to increase the frequencies of B-type of κ-casein through the appropriate selection and breeding schemes, by selecting sires bearing κ-casein B gene.

It was concluded that, Genotyping of milk proteins, such as k-casein gene is extremely important for selection practice to improve industrial milk production and could be used in selection criteria for dairy sires.

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