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Analysis of Selection Effect Based on Kappa Casein Gene on Milk Yield Production of Iranian Sarabi Cattle Breed Using Stochastic Simulation

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Abstract: PCR-RFLP was used to genotype 87 Sarabi native cattle of north-western Iran for A and B alleles of kappa casein gene. A 350 bp length of exon 4 and intron 4 was amplified and digested with *HinfI* endonuclease. Samples were loaded on agarose gel (2%) and genotyped under UV light. Allele frequency of desirable B allele was 0.57. Stochastic simulation was used to generate milk yield trait for a population of 4950 females and 50 males for 15 overlapping generations. Population parameters included 1100 and 436 kg for average milk yield and phenotypic deviation, respectively; with heritability of 0.27. Additive and dominance effects of Kappa Casein gene were considered as 187.63 and 50.37 kg, respectively. Two methods were considered for selection of males based on the first phenotypic record of their dams (PAS) or molecular information of each male, individually (GAS). Females were always selected on their first phenotypic record. Although, there was a significant difference between polygenic and major gene genetic response between two methods after the 5th generations, but there was no significant difference for the sum of polygenic and major gene response. After 15 generations of selection there was no significant difference between inbreeding coefficient under two methods. Selection plan for males based on one single major gene had no advantage over the conventional selection based on dam record in native Sarabi breed.

Key words: Simulation, Kappa casein, PCR-RFLP, phenotypic assisted selection, genotypic assisted selection

INTRODUCTION

To date, most genetic progress for quantitative traits in livestock have been made by selection on phenotype or on estimates of breeding values derived from phenotype without any knowledge of the number of genes that affect the trait or the effects of gene. Although, number of genes is unknown there are some candidate genes for quantitative traits and it is already known that some genes have great influence on genetic variations. As the genes with major effects are discovered, it is possible to directly select on the genotype for genetic improvement of traits (Muir and Stick, 1997). This has enabled opportunities to enhance genetic improvement programs in livestock by directly selection on genes or genomic regions that affect economic traits. Many theoretical studies have been conducted over the past several decades to evaluate strategies for the use of molecular genetic information in selection programs. Application of molecular genetic for genetic improvement relies on the ability to genotype individuals for specific genetic loci (Dekkers, 2004). Discovery of several types of DNA markers have allowed for a comprehensive search for associated Quantitative Trait Loci (QTL). One of the observable polymorphic genetic loci is direct markers which code for the functional mutation. Such a marker allow for selection on genotype across the population because of the consistent association between genotype and phenotype (Dekkers, 2004). The impact of using these recent information in selection has been studied either analytically or by means of computer simulation (Abdel-Azim and Freeman, 2002). Gibson (1994) used stochastic simulation to select based on genetic information and his results showed extra response in early generations but less response in later generations. Muir and Stick (1997) investigated the potential relative advantage that direct selection on the genotype adds to

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a breeding value. Their results showed the greatest response in both short and long term with Marker Assisted Selection (MAS). However, when the number of loci was greatly reduced, such that the major gene accounted for a much larger proportion of the genetic variance, the results were similar to that observed by Gibson (1994). Dekkers and Van Arendonk (1998) used optimal control theory to optimize selection on an identified QTL by optimizing weights b in the index of selection. Several putative QTL have been found associated with type and production traits in dairy cattle. Comparing results across all studies would suggest that QTL affecting milk production traits in cattle are segregating on chromosome 3, 6, 14 and 20 (Sonstegard *et al*., 2001). Kappa Casein is a protein in mammalian milk whose gene is located on chromosome 6 of cattle and it has two common genetic variants of A and B. This gene is considered as a candidate gene for milk yield and component. The A variant has a Thr and Asp at positions 136 and 148, while the B variant has Ile and Ala in these positions. These two alleles determine great differences in milk, casein, protein and fat yields as well as cheese making properties such as coagulation time and curd firmness, with a superiority for cheese production of the k-casein BB compared to k-casein AA milk (Lodes *et al*., 1996). The effect of B allele on increasing of protein yield (Ng-Kwai-Hang, 1998; Cowan *et al*., 1992; Tsiaras, 2005), protein percentage (Van Eenennaam and Medrano, 1991) and casein percentage (Ng-Kwai-Hang, 1998; Bobe *et al*., 1999) and decreasing of fat percentage (Cowan *et al*., 1992) was also reported. The aim of this study was to determine the allelic frequency in polymorphic site of exon 4 of k-casein gene in Iranian Sarabi native cattle, opening the possibility for the investigation of molecular gene effects on breeding programs of this breed.

MATERIALS AND METHODS

Iranian native Sarabi dairy cattle is spread in the Northwest of Iran. PCR-RFLP was used to genotype 87 animals of Sarabi cattle for A and B alleles of kappa casein gene. A 350 bp length of exon 4 and intron 4 was amplified and digested with *HinfI* endonuclease. Samples were loaded on agarose gel (2%) and genotyped under UV light. Also, stochastic simulation was used to generate milk yield trait for a population of 4950 females and 50 males for 15 overlapping generations. Population parameters included 1100 and 436 kg for average milk yield and phenotypic deviation, respectively and heritability of 0.27 was used. Additive and dominance effects of Kappa Casein gene were considered as 187.63 kg and 50.37 kg, respectively (Cowan *et al*., 1994). Mean of Breeding Values (BV) in the base population was considered null with additive variance $V_A = h^2 * V_P$, which V_P is phenotypic variance. Polygenic variance was computed by subtracting major gene and additive variances. Polygenic breeding values in the base population were simulated by multiplying a random number of normal distribution (Z~ (0, 1)) and polygenic standard deviation (S_{pg}). These values for the next generations were simulated by Eq. 1 and 2.

$$PgBV = \frac{PgBVSire + PgBVDam}{2} + MS \quad (1)$$

$$MS = \sqrt{\frac{2 - FSire - FDam}{4}} * Z * Spg \quad (2)$$

Major gene breeding values were simulated based on additive and dominance effect of kappa casein gene extracted from Cowan *et al*. (1994) and allelic frequencies of each generations. These values for AA, AB and BB kappa casein genotypes were equal to 2qα, α(q-p) and-2pα (Falconer and MacKay, 1996). Phenotypes were determined by adding PgBV, MgBV and a random environmental effect (Eq. 3 and 4).

$$Phenotype = Mean + PgBV + MgBV + Z * Se \quad (3)$$

$$Se = \sqrt{Vp(1 - h^2)} \quad (4)$$

Generations were overlapped and base population had 4950 females and 50 males. Females were kept for 5 lactations at most and males were kept until 4 years in the herd. Culling rate for females and males were considered 5 and 20%, respectively. Females were always selected on their first phenotypic record. Two methods were considered for selection of males based on the first phenotypic record of their dams (Polygenic Assisted Selection; PAS) or individual molecular information of each male, (Genotypic Assisted Selection; GAS). The first mating was random and then population was simulated for 15 generations with 30 iterations.

RESULTS

According to k-casein sequence (GenBank X14908); there are two restriction sites for HinfI enzyme (GAnTC) in the 350 bp fragment. The permanent site leads to a restriction of the 350 bp product to two fragments with a length of 266 and 84 bp. If a polymorphic site exists, the 266 bp can be cut into 132 and 134 bp. Thus, homozygous

AA genotype digested with *HinfI* endonuclease enzyme showed 3 fragments with the length of 134, 132, 84 bp, heterozygous AB with 4 fragments of 266, 134, 132, 84 bp and homozygous BB genotype 2 fragments with 266 and 84 bp (Fig. 1). The B allele frequency was calculated to be 0.57 by counting method as

$$p = \frac{2(BB) + (AB)}{2N}, \; q = 1\text{-}p;$$

where:

p = the gene frequency of allele B and
q = the gene frequency of allele A.

Although, there was a significant difference between polygenic and major gene response in two methods, after the 5th generations, but there was no significant difference between total breeding values (Fig. 2 and 3).

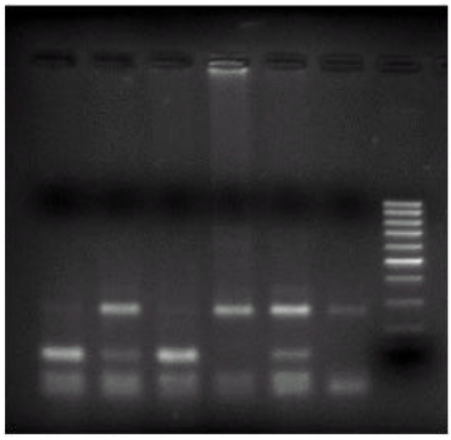

Fig. 1: k-casein genotypes. Columns (left to right) 1, 3 genotype AA; columns 2, 5 genotype AB and columns 4, 6 genotype BB. M, marker 100 bp

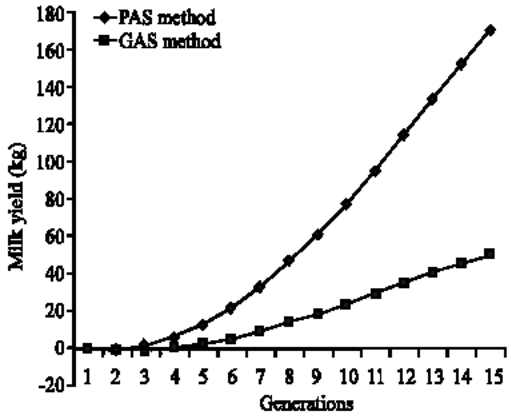

Fig. 2: Polygenic breeding value responses during 15 generations

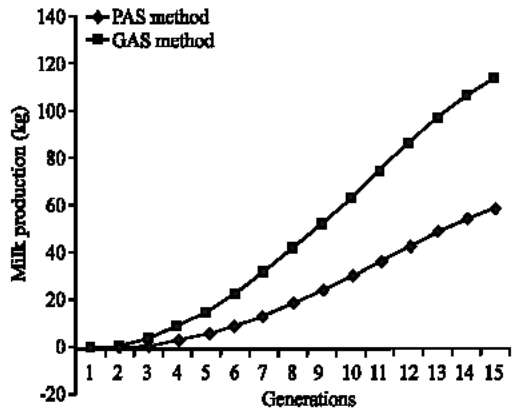

Fig. 3: Major gene breeding value responses during 15 generations

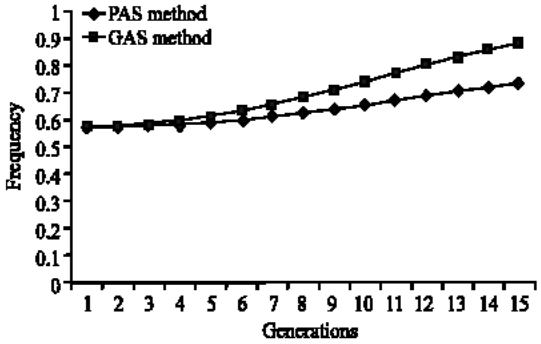

Fig. 4: Differences in major gene frequency in PAS and GAS methods

It seems that major gene trends to be fixed sooner in GAS method compared to PAS method (Fig. 4). After 15 generations of selection there was no significant difference between inbreeding coefficient between two methods.

DISCUSSION

The frequency of B allele of k-casein gene in Holstein breed ranges from 0.06 to 0.57 (Tsiaras *et al*., 2005; Bobe *et al*., 1999; Ron *et al*., 1992; Cowan *et al*., 1992; Van Eenennaam and Medrano, 1991). The highest frequency is observed in Brown Swiss and Jersey with 0.67 and 0.86, respectively. Frequency of this allele in Norwegian cattle breeds and Northern regions of Europe is low (Lien *et al*., 1999). As it has been shown in Fig. 2, response to major gene in GAS is much higher than PAS method. Although, superiority of major gene response in GAS increased in early years of using major gene, it seems eventually decreased after fixation of favorable allele, which may be due to reduction in variance of major gene

(Abdel-Azim and Freeman, 2003). The inferiority of the polygenic response of the GAS method was clearly demonstrated for all 16 generations which is similar to Abdel-Azim and Freeman (2003) results. It was also reported that phenotypic selection scheme increased average polygene gene frequency faster than combination of major gene and phenotype (Muir and Stick, 1997), but the response in total genetic gain was faster with marker assisted selection than with traditional selection and persisted over several generations (Schulman and Dentine, 2005). Muir and Stick (1998) showed that selection strategies which aim to fix the major gene as quickly as possible achieve less response to selection than traditional selection based on phenotype, both in the short and longer term. The optimal program is not to fix the gene as rapidly as possible otherwise animals with many favorable alleles will also be discarded. Simulation study has showed that for heritability of 1 and 10%, the rate of inbreeding was always greater for combination of phenotypic and major gene information than phenotypic strategy; but at a heritability of 40%, differences in inbreeding were minor (Muir and Stick, 1997).

CONCLUSIONS

The aim of this study was to investigate the superiority of major gene information in breeding programs of Iranian native Srabi cattle. It seems that selection plan for males based on one single major gene had no advantage over the conventional selection based on dam record in this breed. It might be due to the location of k-casein gene in a cluster of 200 bp on chromosome 6 or the higher effects of polygenes which influence milk production. It is strongly recommended before this information can be applied in commercial programs, economic trait loci be identified, validated and characterized for improving genetic gain. Also, it may be considered as a separate trait with its own relative weight proportional to the values of the overall genetic variance for the trait. As casein genes are located on the same region, it seems that considering haplotype allele effect might have more precise effect rather than individual gene effect.

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