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Analysis of Kappa Casein Gene Polymorphism by PCR-RFLP in Buffalo Population in Khouzestan Province

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Abstract: Caseins are a family of milk proteins that approximately constitute 80% of total milk proteins. They are existing in several molecular forms (alpha S_1, alpha S_2, beta and kappa) with variant alleles of each. The A and B are the most common in cattle breeds. Allele A has threonine (ACC) and aspartic asid (GAT) amino acid at position 136 and 148, respectively by means of point mutation, isoleucine (ATC) substitutes threonine and aspartic acid is substituted by alanine (GCT) and variant A changes to variant B. In this study, the PCR-RFLP method was used for differentiating buffalo population in Khouzestan Province k-CN allels. In order to determaind the level of polymorphism, blood sample were collected from 86 buffalos in Shadegan, Ahwaz, Sosangerd, Dezfoul and Shoshtar cities. DNA extraction was based on Boom method and exon 4 of the kappa casein gene was amplified by specific set of primers for this gene to produce a 453 bp fragment. The amplified fragment were digested with Hinf I restriction endonuclease and then subjected to electrophoresis separation in ethidium bromide-stained 2.5% agarose gel. The results were revealed all buffalo samples were monomorph and genotyped as BB. In this breed, the B allele is only existed, which is reported to be favorable for milk quality and can use for breeding strategies of dairy animals.

Key words: Polymorphism, kappa casein, buffalo, milk, PCR-RFLP, Khouzestan

INTRODUCTION

Milk and milk products is important for feeding because supply nutrients, energy, high quality protein, vitamins and minerals requarments. The composition of the milk of different species varies in the percentages of these constituents. All milks contain the same kinds of constituents, but in varying amounts (Otaviano *et al*., 2005).

Milk proteins are usually divided into two fractions. The soluble fraction, named whey protein, constitutes the α-lactoalbumen β-lactoglobulin. The insoluble fraction, named whole casein, constitutes 4 different caseins (alpha S_1, alpha S_2. Beta and kappa caseins) (El-Rafey and Darwish, 2007; Rachagani and Gupta, 2008; Unsal *et al*., 2008). The casein fraction of milk proteins significantly influences the composition and physic-chemical properties of the milk (Grosclaude, 1988).

Kappa casein is one of the most important milk proteins, controlled by means of a gene with 5 exon and 4 intron. Kappa casein gene is one of 4 groupes of major gene in milk casein fraction, with 19,800 dalton molecular weight and 169 amino acids, exist on chromosome No. 6 in bovine and chromosome No. 4 in sheep and goat. Mount of produced cheese and also milk permanent (viable) out of refrigerator directly dependent on properties of milk casein. Variant B of kappa casein gene result by means of point mutation (T/C) at position exon 4, due to increasing efficiency production cheese of milk (Henderson, 1971; Medrano and Aguilar, 1990). In sperm catalogs, BB or AB genotypes indicator ideal genotypes of milk production for use in cheese making (yielding) factories that due to decreasing coagulation time and increasing curdling stability of milk. In this reason, domesticator that their herd's milk are used for cheese making, use sperms with BB or AB genotypes until increase B allele frequency in herd and gradually increase ideal genotype of this gene in their domestic (Alipanah *et al*., 2008; Dayem *et al*., 2008).

Buffalo yield about 12% of milk yielding in the world. In recent years, buffalo's milk and it's products specially mozzarella are favorite and dairy buffalo rear in unnative area. Therefore, it's milk proteins and genes controlle them, are very Important also, selection on molecular markers is more reliable than other methods (Othman, 2005).

The objective of this study was to determine the genotypes and allelic frequency for kappa casein by use of PCR-RFLP technique as a fast efficient and low cost method in buffaloes of Khouzestan Province.

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MATERIALS AND METHODS

Blood sample collection: In this study, blood sample were collected from 86 buffaloes in Shadegan, Ahwaz, Sosangerd, Dezfoul and Shoshtar cities. The 5 mL blood were collected by needle punctionure with EDTA of the jugular vein.

DNA extraction: Genomic DNA was extracted from whole blood by kit DIAtom DNA prep 100. The quality and concentration of extracred DNA was assessed by electrophoresis on 0.8% agarose gel.

Polymerase chain reaction: The primers used for the amplification of the k-CN gene fragments were already reported by Barroso *et al*. (1998) and Riaz *et al*. (2008) with the following nucleotide sequence:

KF5'- TGTGCTGAGTAGGTATCCTAGTTATGG-3'
KR5'- GCGTTGTCTTCTTTGATGTCTCCT-3'

Final volume of 25 μL containing: 1X Taq polymerase buffer, 200 μm dNTPs, 1.5 μm $Mgcl_2$, 10 pico moles of each primers, 1 U Taq DNA polymerase and 100 ng DNA.

The amplification was carried out in thermal cycler with the following amplification conditions: 94°C for 5 min (initia denaturation) 94°C for 1 min, 65°C for n1 min and 72°C for 2 min with a final extention step of 72°C for 10 min upto 35 cycles.

RFLP (Restriction Fragment Length Polymorphism) technique: PCR products were digested by Hinf I restriction enzyme. Final reaction volume of 30 μL containing: 10 μL of each PCR product, 2 μL of buffer 10X,1 μL Hinf I enzyme (10 unit μL^{-1}). After incubation of the reaction mixture at 37°C for 12-16 h, the resulting digested fragment were analyzed by electrophoresis on 2/5% agarose gel stained witht ethidium bromide in 1X TBE as the running buffer at 80 V for approximately, 70 min the bands were visualized under UV-light to observe the polymorphic locus by the size change of DNA fragments.

RESULTS AND DISCUSSION

PCR amplification using primers KF and KR yielded a 453 bp DNA fragment of k-CN gene. PCR products from this population, after being digested with Hinf I that identify changes in kodon 148 (aspartic acid is substituted by alanine) generated two fragments, 426 and 27 bp indicate genotype BB. Analysis of this results depicted the existence of only B allele (Fig. 1 and 2).

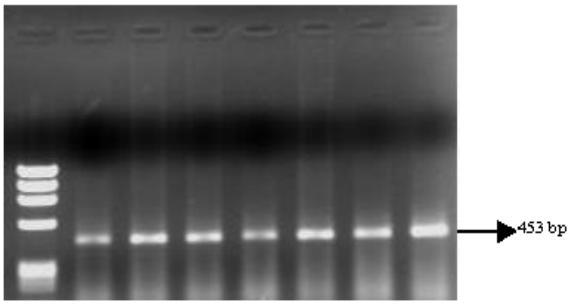

Fig. 1: The 453 bp PCR products of kappa casein gene

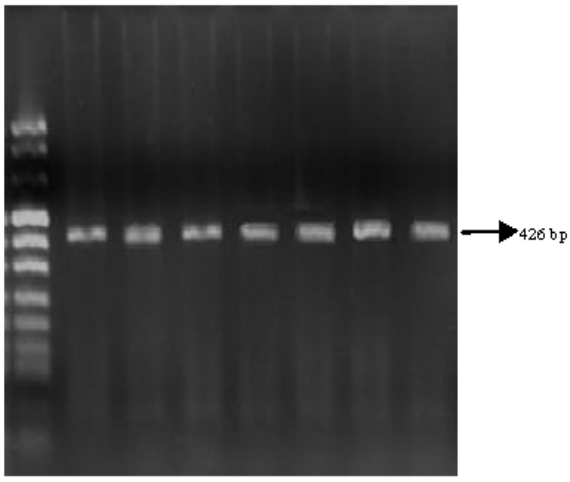

Fig. 2: PCR amplified K-casein products digested with Hinf I. Restriction digested PCR products showed two fragmants of 426 and 27 bp

The observations for kappa casein are similar to the findings of Riaz *et al*. (2008) who noted one allele B in Nili-Ravi breed of Pakistan. Raj *et al*. (2008), El-Rafey and Darwish (2007) and Otaviano *et al*. (2005) also found monomorphism (BB) for this gene in buffalos. However, Patel *et al*. (2007) found two alleles A and B for k-CN locus in Murrah, Surti and Pandharpuri breeds of buffalo.

Monomorphic form BB of k-CN is responsible for higher yield in cheese making as well as milk and milk protein yield. The cheese production can be increased by 10% if milk from cows of genotype BB of k-casein is used (Marziali and Ng-Kwai-Hang, 1986).

The present study is the first report on k-CN genotyping of buffalos in Khouzestan Province that indicate uniform and homozygous population for k-CN B allele.

CONCLUSION

The PCR technique amplified a DNA fragment of CSN3 gene with 453 bp. The results of the RFLP analysis showed two fragments 426 and 27 bp after restriction with

enzyme with Hinf I that identify changes in kodon 148 (aspartic acid is substituted by alanine). This result indicate homozygous, with genotype BB. Analysis of this results depicted the existence of only B allele.

It was not possible to assess how much the buffalo kappa-casein gene affects milk yield and its components, since all animals studied were monomorphic for that gene as determined by PCR-RFLP.

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