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Lactoferrin Gene Polymorphism of Holstein Cows in Isfahan Province

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ABSTRACT

A total of 404 Holstein cows from five dairy herds in Isfahan province were used to obtained polymorphism of bovine lactoferrin (LTF)gene for a possible genetic marker information. Two alleles A and B were found in the examined population. The frequency of A-allele ranged from 0.775 to 0.831, while frequency of B-allele 0.169 to 0.225. The alleles controlled the occurrence of two genotypes AA and AB,with frequency of 0.606 and 0.394 respectively.Statistical analysis showed that there was no Hardy-Weinberg equilibrium between the observed and expected distribution of LTF genotypes.It was found that polymorphism existed in intron 6 region of bovine LTF gene, which suggested that this polymorphism could be associated with somatic cell count (susceptibility/resistance mastitis).

Key words: Bovine lactoferrin gene, polymorphism,PCR-RFLP, Holstein cows, mastitis.

INTRODUCTION

Among health or disease traits in dairy cattle, mastitis is one of the most costly disease and therefore a potential trait to be included in breeding objectives[1]. Results of the numerous studies indicatedthat mastitis resistance is a very low heritable trait andtherefore direct selectionis very difficult to improve this trait[2, 3]. Somatic cell count (SCC) of milk is closely linked to the magnitude of the inflammatory process and it is also a good diagnostic tool that allows early detection of both subclinical and clinical mastitis [4, 5].Accumulating results showed heritability of SCC is greater for clinical mastitis and there is average genetic correlation 0.7 between SCC and clinical case [4, 6]. Hence, breeding for resistance to this disease can be performed by indirect selection the cows using SCC of milk. Wojdak-Maksymiec*et.al.*indicated that there is an association between bovineLTF gene polymorphism and SCC [5]. LTFis known as “the red protein”and it was firstly identifiedand reported in 1939 in milk whey [7]. Bovine LTF is an iron-binding glycoprotein and a member of a transferrin familythat found in milk and other exocrine secretions including saliva, tears, bile, urine, semen, vaginal fluids, nasal and bronchial secretion and blood plasma [8]. This gene is located on the BTA22 chromosome and consists of 17 exons (ranging from 82 to 225bp), 1122 bp of promoter region and spans approximately 34.5 kb of genomic DNA [9, 10]. Lohuis *et.al*. suggested that bovine LTF might have potential in the treatment of bovine mastitis [11]. Many studies recently showed that this glycoprotein serves an important function in the natural defence mechanism of the mammary gland [7, 12].This suggests that the glycoprotein playsimportant physiological roles in inflammatory, particularly during mastitis event, and it would be a potential candidate gene for imparting resistance mastitis in dairy cows. The purpose of this study was to estimate allele and genotype frequencies of bovine LTF gene polymorphism in Holstein dairy cows, and test genotypic for Hardy-Weinberg equilibrium.

MATERIALS AND METHODS

In this experiment, blood samples were obtained from 404 Holstein dairy cows that randomly selected among about 10000 cows from five intensive herds in Isfahan province. Genomic DNA was extracted from the whole blood using

the salting out method [13].The LTF genotypes were identified with the PCR –RFLP technique. The isolate DNA was used for PCR amplification of the LTF gene fragment of 301 bp with the use of the selected primer. Sequences of the primers that were used in PCR were reported previously by wojdak-Maksymiec*et.al*. [5]. Sequences of LTFR and LTFF were 5'-CAG GTT GAC ACA TCG GTT GAC-3'and5'-GCC TCA TGA CAA CTC CCA CAC-3', respectively.The PCR mixture contained 2 µL of DNA, 2 µL 10X PCR buffer, 10 pmoL of each primer, 1.5 mM $Mgcl_2$, 200 µM dNTP, 1 unit Taq DNA polymerase and sterilized distilled water to make a final volume of 20 µL. Conditions for PCR were 94˚C for 2 min, followed by 32 cycles of 94˚C for 60 s, 61˚C for 45 s and 72˚C for 60 s. The final step was at 72˚C for 5 min. PCR products were digested with *Eco*RI enzyme (Fermentas Co.) which were used for determination of LTF A and B alleles. Eight microliters PCR products was digested with five units of enzyme in 20 µL of reaction at 37˚C for 6 h for RFLP of the LTF gene. Restriction fragments were analysed electrophoretically in 2% agarose gel in TBE buffer for 2 h, and the genotype bands were visualized under UV light.

RESULTS AND DISCUSSION

In this experiment, PCR-RFLP method was utilized as a useful approach for LTF genotype marking and it could be considered as selection criterion in dairy cattle population. Investigation of the polymorphism for this gene first reported by Seyfert and Kuhn, that found two alleles A and B with frequencies of 0.755 and 0.245 respectively [10].The primers used in this study were similar to those utilized by Wojdak-Maksymiec *et.al*. in Polish BlackandWhite cattle [5]. The A and B alleles of the LTF gene were identified based on amplification of specific primer (LFTR and LFTF), followed by digestion with the restriction enzyme *Eco*RI (Fig. 1).

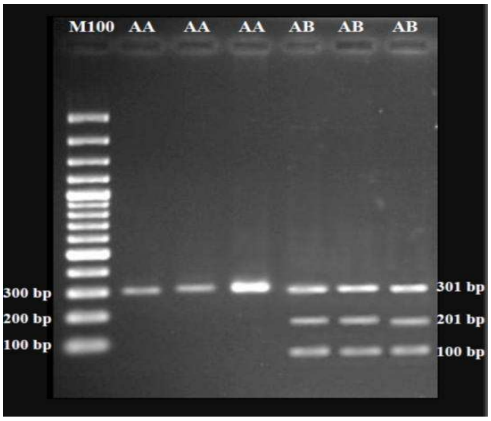

Figure 1: Gel electrophoresis of PCR product after digestion with *Eco*RI restriction enzyme. From left to right; lane 1 = molecular size markers; lanes 2, 3 and 4 = AA genotype (301 bp) and lanes 5, 6 and 7 AB genotype (301, 201 and 100 bp).

The frequency of A allele ranged from 0.775 to 0.831 and the frequency of B allele was 0.169 to 0.225 (Table 1). Two genotypes AA and AB of the LTF gene were observed in this experiment. Their frequencies were 0.606 and 0.394, respectively, whileBB genotype was not detected. Genotype AA was characterized by single 301-bp fragment and AB genotype was determined by the presence of three restriction fragments of 301, 201 and 100-bp.The frequency of the AA genotype in the all herds was higher than AB genotype.

The result of χ^2 test indicated that statistically significant (p□0.01) deviation were found in the studied population between the observed and expected distribution of LTF genotypes, according to the Hardy-Weinberg equilibrium (Table 2).

Table1. Frequencies of genotypes and alleles of the LTF gene in Holstein dairy cows

Herd	N	Genotypes		Alleles	
		AA	AB	A	B
1	65	0.550	0.450	0.775	0.225
2	96	0.570	0.430	0.875	0.215
3	76	0.618	0.382	0.809	0.191
4	83	0.663	0.337	0.831	0.169
5	84	0.620	0.380	0.810	0.190
Total/Average	404	06064	0.3936	0.803	0.197

Table2. Frequencies of LTF genotypes in the analysed population

LTF genotype	Observed frequency (%)	Expected frequency (%)	Chi-square
AA	60.64	64.51	0.93857
AB	39.36	31.62	7.65472
BB	0.00	3.87	15.63000
Total	100.00	100.00	24.22329

Chi-square= 24.22329; df= 1; P ≤ 0.0001

Significantly more heterozygotes AB genotypes were find in relation to the expected rate of heterozygotes, whereas there were not BB homozygotes in comparison than the expected rate.Previously, it was stated that LTF gene can control the broad-spectrum antimicrobial activity, especially against coliform bacteria, such as *Escherichia coli*, which cause severe mastitis in dairy cows. Several studies have investigated regards association between bovine LTF gene polymorphism and SCC of milk [5, 14, 15].According to the Wojdak- Maksymiec *et.al*. there is an association between bovine LTF gene polymorphism in the intron 6 region and SCC in Polish Blackandwhite dairy cows, that reported animals with AA genotypes are associated with lower SCC than AB groups [5]. However, Sender *et.al*. provided contrary results and reported genotype BB animals showed the lowest SCC than the other groups [14]. Because of the low frequency of the BB genotype, investigation on this polymorphism need to be continued. Another investigation for polymorphism of bovine LTF gene in the same region has been reported by Šrubařová and Dvořák, who showed two genotypes AA and AB of LTF gene with frequency 57.14% and 42.86% for AA and AB respectively. And claimed there is no significant difference between bovine LTF gene polymorphism and SCC of milk [15].

CONCLUSION

In summary, we showed that dairy cattle LTF gene polymorphism is distinguishable by examining LTF/*Eco*RI, and this gene could be as a marker for susceptibility/resistance to mastitis.

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