The Journal of Animal & Plant Sciences, 22(1): 2012, Page: 25-28
ISSN: 1018-7081

DETERMINATION OF PIT-1/*HINF1* POLYMORPHISM IN HOLSTEIN AND NATIVE EAR CATTLE RAISED AS GENETIC RESOURCE IN TURKEY

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ABSTRACT

Pit-1 gene has been identified as the pituitary specific transcription factor that regulates the expression of the growth hormone (GH) and prolactin (PRL) genes in the anterior pituitary. The use of polymorphic markers in breeding programmes could make selection more accurate and efficient. A total of 252, 181 Holstein cows and 71 native East Anatolian Red (EAR) cattle, were genotyped for polymorphism of Pit-1/*Hinf1* gene by the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) methods. In the Pit-1 gene, the frequency of AA, AB, and BB genotypes was 14, 54, and 32% for EAR, and 4, 31, and 65% for Holstein, respectively. The frequency of A allele was 41% for EAR, and 20% for Holstein. Both populations were in Hardy-Weinberg equilibrium.

Keywords: Pit-1 gene, Polymorphism, PCR-RFLP, Genetic resource, Cattle.

INTRODUCTION

Milk yield, growth and carcass traits are under the controll of multiple genes in livestock. Selection of animals with higher yield bears great significance to breeders and farmers. Current technologies enable scientists to improve on the accuracy and efficiency of traditional selection methods by applying genetic markers through marker-assisted selection. RFLP polymorphisms are significantly associated with certain traits of economic importance and offer useful tools for breeding and improvement of livestock productivity(Yu *et al.* 1995; Renaville *et al.* 1997a; Woollard *et al.* 2000; Wu *et al.* 2005).

Pituitary-specific transcription factor is responsible for pituitary development and hormone expression in mammals (Cohen *et al.* 1997). It was shown to control transcription of the growth hormone, prolactin (Nelson et al. 1988; Mangalam et al. 1989), the thyroid-stimulation hormone, ß-subunit (Simmons et al. 1990; Steinfelder *et al.* 1991), the GHRH receptor genes (Lin *et al.* 1992), and the Pit-1 gene itself (Rhodes *et al.* 1993). Pit-1 is a member of the POU (Pit-Oct-Unc) domain containing proteins, which is a group of transcriptional regulators that have a critical role in differentiation and proliferation of cells (Mangalam *et al.* 1989).

For the Pit-1 gene, several polymorphisms have been reported at different sites including peptide-encoding regions. These polymorphism in the Pit-1 gene lead to the absence of growth hormone, pituitary hypoplasia (Li *et al.* 1990) and congenital hypothyroidism in mice dwarfism, and prolactin deficiency in humans (Pfaffle *et al.* 1992). In domestic animals, cattle, sheep and goat, Pit-1 gene was located on chromosome 1q21-q22 (Woollard *et al.* 2000), whereas in porcine on 13q46. Genetic variations of cattle and porcine Pit-1 gene have been reported to be associated with economic traits and production performance (Yu *et al.* 1995; Renaville *et al.* 1997b; Stancekova *et al.* 1999; Sun *et al.* 2002; Zhao *et al.* 2004; Kai *et al.* 2006). Moreover, QTL (Quantitative Trait Loci) detection revealed that the Pit-1 gene, on cattle 1q21-q22 had an effect on animal production (Woollard *et al.* 2000) therefore making Pit-1 to be a potential candidate gene for economic traits.

East Anatolian Red (EAR) is an important indigenous genetic resource adapted to the harsh climate, poor quality pasture in uplands of East Anatolia. EAR cattle is a dual purpose breed and is raised for meat and milk. Common color of the EAR is light red, but color varies from light to dark red. Average mature weight ranges from 250-300 kg. The average lactation period and milk yields are 170 days and 700-800 kg with a 5-8% fat content (Soysal *et al.* 2004). Finding genetic polymorphisms is constitutes the first setp towards it genetic improvement for a subsequent incorporation of genetic superiority into effective breeding plans for its propagation. The aim of this study is to discover comparative Pit-1 gene polymorphisms in EAR and Holstein.

MATERIALS AND METHODS

Blood samples were collected in a 10 ml vacuum tube containing K_3EDTA, from the left jugular vein from 71 East Anatolian Red animals maintained as genetic

resource in Erzurum, and 181 Holsteins reared at Dogan Organic Products Company in Gumushane in Turkey. Genomic DNA was extracted from whole blood samples using the Purgene kit (Gentra Systems, Plymouth, MN, USA) and stored at 4 ^{o}C.

For Pit-1 gene, two sets of primers were designed from the National Centre for Biotechnology Information (NCBI) GenBank sequences (accession no: NW_001493841.2) by the Primer3 program (Rozen and Skaletsky, 2000) and the Pit-1 gene specific forward and reverse primers (5'- ACT CGC TAT TAC ACA ATA GGA GAG CCT-3' and 5'-TCC TGC CAA CTC CTC ACC TCC C-3') respectively which were used to amplify a 260 bp fragment in PIT-1 gene.. Amplification reactions were performed in a final volume 30-µl containing 1 µM of each Primer, 1 µl dNTP (D7595: Sigma, St. Louis, MO, USA), 0.5 U of Taq DNA Polymerase (D1806: Sigma, St. Louis, MO, USA), approximately 150 ng of template DNA, 3µl of 10x PCR Buffer (100mM Tris-HCl, pH 8.3, 500mM KCl, 15 mM $MgCl_2$ and 0.01% gelatin), 1µl of 25 mM $MgCl_2$ and ddH_2O. PCR amplifications were performed in 5 min at 94 ^{o}C, 30 cycles of 45 s at 94 ^{o}C, 60 ^{o}C and 72^{o}C, which was followed by final extension at 72 ^{o}C for 5 min. Products with completed amplifications were stored at -20 ^{o}C until the next step. To genotype animals for RFLP, 7 µl PCR reaction mix was used for HinfI enzyme digestion which was performed in 15µl volume in 0.2 ml-sterilized eppendorf tubes. Each 15 µl-digestion mix was electrophoresed in 2.5% agarose gel at 40V for 2.5 h and DNA was visualized by staining with ethidium bromide under UV light. A standard DNA marker (P1473: Sigma, St. Louis, MO, USA) was used.

For each cattle breed, Pit-1 allele frequencies were determined by gene counting. The Chi-square (χ^2) test was used to check whether the populations were in Hardy-Weinberg equilibrium or not.

RESULTS AND DISCUSSION

RFLP polymorphism within the bovine Pit-1 gene detected by HinfI restriction enzyme are illustrated in Figure 1, showing the fragments obtained for the Pit-1/*Hinf1* polymorphism: 190 and 70 bp for the BB genotype, 260, 190 and 70 for the AB and 260 for the AA.

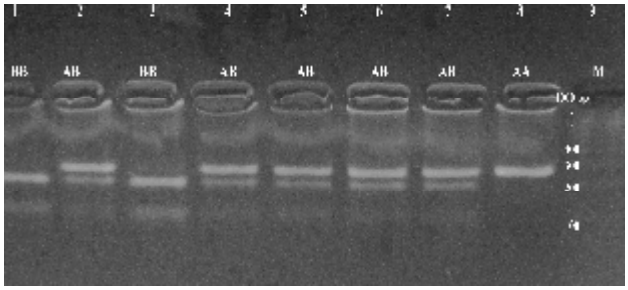

Fig. 1: Representative results of Pit-1/HinfI analysis detected by agarose gel electrophoresis (1 and 3 lines; BB, 2, 4, 5, 6, and 7 lines; AB and 8 line; AA, M: DNA marker).

Table 1 shows the frequency distribution of allelic variations for Pit-1 gene in EAR and Holstein cattle. Differences among breeds for Pit-1 genotypic frequency equilibrium were highly significant ($p<0.001$). In genetic balance test, Pit-1 genotypes were found to be in equilibrium within and among breeds ($p>0.05$).

Table 1: Distribution of the allelic and genotypic frequencies of the Pit-1 gene in the two breeds of cattle and the statistical test results for Hardy-Weinberg equilibrium

Breed	N	Genotypes			Alleles		Hardy-Weinberg equilibrium X^2 test
		AA	AB	BB	A	B	
EAR	71	0.14 (n=10)	0.54 (n=38)	0.32 (n=23)	0.41	0.59	0.82 ns
Holstein	181	0.04 (n=8)	0.31 (n=56)	0.65 (n=117)	0.20	0.80	0.15 ns
Total	252	0.07 (n=18)	0.37 (n=94)	0.56 (n=140)	0.26	0.74	0.17 ns

n: observed number, Independent X^2 test=30.65 ($p<0.001$), ns: non-significant.

In the Pit-1 gene, the frequencies of AA, AB, and BB genotypes were 14, 54 and 32% for EAR, and 4, 31 and 65% for Holstein, respectively. The frequency of A allele was 40,8% for EAR, and 19,9% for Holstein. The frequency of Pit-1/*Hinf1* B allele was higher in Holstein than EAR.

Allelic frequencies of Pit-1 gene was estimated to be 15.5 and 84.5% for A and B, respectively, which was consistent to the data of Italian Holstein breed (Renaville *et al.* 1997b). Zwierzchowski *et al.* (2002) estimated the Pit-1 A allele frequency to be 0.45 in Angus, 0.26 in Holstein, 0.21 in Hereford, 0.28 in Gelbvieh, 0.10 in Brahman, 0.25 in Polish and 0.95 in Gry cattle. Misrianti *et al.* (2010) revealed that the genotype frequencies of AA, AB, and BB were 0.02, 0.44, and 0.53 in Friesian Holstein cows respectively, and

frequencies of A allele and B allele in Pit-1 were 0.25 and 0.75, respectively.

There are several reports on genotypic frequency of the AA genotype in different breeds of cattle. The frequency of the AA genotype for Angus beef cattle was reported as 0.11 (Zhao *et al.* 2004), for Polish Black and white cattle as 0.09 and 0.052 (Zwierzchowski *et al.* 2002; Dybus *et al.* 2004), which all of them were higher for Holstein in the present study, but not EAR. However, in contrast the frequency of AA gene for Italian Holstein-Friesian bulls was 0.022 (Renaville *et al.* 1997b) which were lower than the present study. Genetic disequilibrium found in the present study showed that the selection could be effective for BB genotype in Holstein, but not EAR cattle.

Screening favorable alleles for selection at the DNA level provides an ideal tool for marker-asisited selection. RFLP polymorphism within the bovine Pit-1 gene was first detected with HinfI nuclease by Woolard *et al.* (1994). Since many reports are available regarding the effect of Pit-1 gene polymorphisms on economic important traits (Yu *et al.* 1995; Sabour *et al.* 1996; Renaville *et al.* 1997b; Stancekova *et al.* 1999; Sun *et al.* 2002 and Zhao *et al.* 2004; Kai *et al.* 2006; Lan *et al.* 2007, 2009; Reza *et al.* 2010). Sabour *et al.* (1996) showed that allele A in Pit-1 locus positively affected milk production traits in Friesian cattle. An allelic frequency of 0.18 was significanctly superior over allele B for milk and milk protein yields and body conformation traits in Italian Holstein Friesian cattle. Reza *et al.* (2010) reported similar polymorphism of Pit-1 gene and its effect on the growth traits in Nanyang cattle. It is clear that cows with genotype BB had remarkable growth performance, that could be used for breeding new lines of beef cattle (Renaville *et al.* 1997a). These results could help improving native EAR cattle. These suggestions may be instructional for the early breeding selection

Acknowledgements: The author sincerely thanks Erdogan Sezgin from East Anatolian Agricultural Research Institute in Erzurum, and Dogan Organic Products Company in Gumushane for their collaboration in sampling procedure.

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