



bGH/*AluI* and CSN3/*HinfI* Gene Polymorphisms in Holstein Cattle

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Abstract

The aim of the study was to determine the gene and genotype frequencies of the bGH/*AluI* and CSN3/*HinfI* gene in the animal material obtained from organic farm by PCR-RFLP method. A total of 248 cattle of Holstein breed were genotyped for the bGH/*AluI* and CSN3/*HinfI* polymorphism. In bGH gene region, the LL, LV and VV genotype frequencies were 0.50, 0.48 and 0.02 respectively and In CSN3 gene region, the AA, AB and BB genotype frequencies were 0.71, 0.23 and 0.06 respectively. Both bGH/*AluI* and CSN3/*HinfI* genotypes were not found to be in equilibrium within the breed. Also, both heterozygosity were found at a high rate as 0.478 and 0.234 and the calculated F_{IS} values were -0.24 and 0.20 respectively.

Keywords: Growth hormone, Kappa-Casein, PCR-RFLP, Polymorphism, Cattle.

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1. Introduction

Due to the development of technological methods and ease of the implementation of DNA-based diagnostic tests and the expansion of the application areas in the molecular genetic field, these methods are widely used in genetic evaluation in animal husbandry. Various molecular markers that can be used at the molecular level of the meat programs of markers providing easiness in the determination and identification of the populations that can be used as genetic resources particularly in the identification of the genetic structure affecting the efficiency in farm animals, in the determination of the animals to be taken into the animal breeding program using quantitative traits within the protection programs, and various molecular genetic methods have been developed enabling the identification of each marker at molecular level [1].

Bovine growth hormone (bGH) is a single chain polypeptide hormone with 22 kDa weight produced in the anterior pituitary gland, and it consists of 191 amino acids. bGH gene was identified on the 19th chromosome in cattle using in-situ hybridization method [2] and contains five exons regions divided into four separate sections, of about 1,8 kb at length.

Several studies have been made on bGH/*Alul* and CSN3/*HinfI* gene polymorphism belonging to various cattle breeds, and the results have been reported (Table 1).

L allele frequency is reported to be high, and V allele frequency is reported to be low in terms of bGH/*Alul* polymorphism across the breeds as a result of the studies conducted (Table1).

Table-1. bGH/*Alul* and CSN3/*HinfI* gene polymorphisms belonging to various cattle breeds

bGH gene			CSN3 Gene				
Cattle Breed	Allele frequencies		Reference	Cattle Breed	Allele frequencies		Reference
	L	V			A	B	
Holstein	0.87	0.13	Echeverri, et al. [3] Fries, et al. [2] Ozdemir [4] Ozkan, et al. [5] Kovacs, et al. [6]	Holstein	0.65	0.35	Echeverri, et al. [3] Fontanesi, et al. [7] Vidovic, et al. [8] Ozdemir and Dogru [9] Botaro, et al. [10] Sitkowska, et al. [11]
	0.86	0.14			0.44	0.56	
	0.90	0.10			0.51	0.49	
	0.84	0.16			0.55	0.45	
	0.93	0.07			0.83	0.17	
Simmental	0.82	0.18	Vasconcellos, et al. [12] Schlee, et al. [13]	Simmental	0.67	0.33	Djedovic, et al. [14]
	0.71	0.29		Simmental crossbred	0.82	0.18	
				Bursa	0.67	0.33	
Jersey	0.76	0.24	Sabour, et al. [15] Lucy, et al. [16]	Sahiwal	0.16	0.82	Mir, et al. [17]
	0.56	0.44					
Brown-Swiss	0.91	0.09	Ozdemir [4] Ozkan, et al. [5] Lucy, et al. [16]	Simmental-Holstein crossbred	0.76	0.24	Trakovicka, et al. [18]
	0.87	0.13					
	1.00	0.00					
East Anatolian Red	0.98	0.02	Ozdemir [4] Ozdemir [19] Ozkan, et al. [5]	Yellow pied	0.55	0.42 (0.03E)	Bartonova, et al. [20]
	0.78	0.22					
	0.84	0.16					
Ayrshire	0.71	0.29	Sabour, et al. [15] Lucy, et al. [16]	Girolando	0.85	0.15	Botaro, et al. [10]
	0.79	0.21					
Turkish Gray	0.89	0.11	Ozdemir [4]	Black pied	0.83	0.17	Alipanah, et al. [21]
				Red Pied	0.69	0.31	
Angus	0.77	0.23	Vasconcellos, et al. [12]	Bestuzhev	0.71	0.29	Sulimova, et al. [22]
				Kalmyk	0.68	0.32	
				Russian Black pied	0.85	0.15	
				Yaroslavl	0.52	0.48	
				Yakut Breed	0.75	0.25	
Charolais	0.72	0.28	Kemenes, et al. [23]	Yellow pied	0.60	0.38	Kucerova, et al. [24]

bGH is reported to play an important role in biological processes such as regulating breast development, lactation, aging, growth and metabolism, and controlling ovulation and sexual behavior in the females [25-27].

In many studies conducted on cattle regarding bGH, researchers reported that it improved growth and increased carcass quality [28-31] was associated with milk yield and quality [6, 15, 16, 32-36] played an active role in carcass composition and quality [13, 37-40] and was associated with reproductive traits [41]. In their study on cattle, Cecchinato, et al. [42] stated that bGH affected the coagulation properties of milk and had an effect on the amount of the casein protein in milk, played an important role in the postnatal growth and general metabolism and also in lactation. Current information shows that bGH has a very important power in nutrient utilization [25] breast development, and growth [43].

In cattle, casein protein located on q31-33 arm of the 6th chromosome is clustered in α S1 (CSN1S1), β (CSN2), α S2 (CSN1S2), ve κ -(CSN3) gene regions which are connected to each other with close gene regions [44, 45].

Frequently seen variants of kappa casein variants are A and B, and their distribution among the breeds varies (Table 1). The relations of these casein loci with various performance elements in a variety of breeds of cattle may also vary. In different studies conducted on CSN3 loci, it was reported that they could be used in indirect selection to increase the milk yield of Holstein cattle, that they can be used as a marker for the milk composition and performance of milk protein genes and, for this purpose, as a valuable mean in milk cattle selection programs [7, 11, 17, 22, 46]. While Djedovic, et al. [14] reported that CSN3 BB type could be used in indirect selection in terms of milk and fat yield and fertility and calf vitality, in the studies conducted in the same way, CSN3 B genotype was reported to be a desirable variant for higher milk yield [8, 9, 24]. In the polymorphism studies on dairy cattle, Curi, et al. [47] reported that with the selection of CSN3 AA or AB type cattle, there would be an

advantage for treatment of milk and fat yield and ultimately milk protein loci could be used as genetic markers in genetic improvement of cattle [10, 14, 18, 20, 21].

The aim of this study is to identify bGH and CSN3 genetic variants by PCR-RFLP method in Holstein cattle raised on organic farms and contribute to the selection of superior productive individuals and creation of breeding programs.

2. Materials and Methods

In this study, the blood of Holstein cattle (n=248), which were organically raised in Gumushane-Kelkit region, was used as the material. Genomic DNA was obtained using commercially available DNA isolation kit (Puregene DNA kit (Gentra Systems, Minnesota, USA)). Quantitative and qualitative controls of DNA were performed using 2% agarose gel electrophoresis before making PCR application in order to replicate the DNA obtained.

In the analysis, 245-bp DNA region was amplified using primers growth hormone (bGH) gene region, Forward: 5'- GTA GGG GAG GGT GGA AAA TG -3, Revers:5' TGA CCCTCA GGT ACG TCT CC -3, designed by Ozdemir [4] and 351 bp DNA region was amplified using primers Kappa-Casein (CSN3) gene region, Forward:5'-ATT TAT GGC CAT TCC ACC AA-3', Revers:5'-ATT AGC CCA TTT CGC CTT CT-3'. PCR amplification conditions were set (the initial denaturation temperature would be the same) as 1 cycle/ 5 minutes at 94°C for bGH and CSN3; after the 2nd denaturation had been set as 30 cycles, elongation cycles were set 45 seconds at 94 °C, 58°C and 72°C for bGH, and 45 seconds at 94 °C, 60°C and 72°C for CSN3 and final extension temperatures were set 1 cycle/7 minutes at 72°C.

A portion of 7µl from each sample of PCR products of bGH and CSN3 genes, the amplification of which took place, was placed in 0.2 ml sterile eppendorf tube; 2-5 U restriction enzyme for the corresponding region, 2-5 µl RE buffer, 5 µl ddH2O were added on and then it was covered by 10-15 µl mineral oil. Then it was placed in the incubator and incubation was carried out for 12 hours at 37°C. After the incubation process, samples were observed under UV light by keeping under 45 voltage in 2% agarose gel for 2,5 hours. Whether the genotype frequencies are in Hardy-Weinberg equilibrium was examined by Chi-square (χ^2) test in GenAlEx 6.5 program [48].

3. Results and Discussion

In the study, by PCR-RFLP, 186 Holstein cattle were genotyped according to the number and size by cutting each PCR product of 245 bp length for bGH gene with AluI restriction enzyme. Genotyping was carried out by taking VV genotype 208/37 bp, LL genotype 157/51/37 and heterozygous LV genotype 208/157/51/37. After 351 bp CSN3 gene region had been amplified using PCR, it was cut using HinfI restriction enzyme. 248 cattle in total were genotyped into four fragments; if the band size was 262/89 fragmented BB; if the band size was 131/131/89 bp fragmented AA and if the band size was 262/131/131/89 bp fragmented heterozygous AB.

Genotype and allele gene frequencies of bGH/AluI and CSN3/HinfI gene polymorphisms are shown in Table 2. Genotype frequencies for 186 head cattle, which were examined for bGH/AluI gene polymorphism, were found as 0.50 for LL genotype, 0.48 for LV genotype and 0.02 for VV genotype. Allele gene frequencies for the cattle examined were calculated as 0.76 for L allele and 0.24 for V allele. Many researchers reported that L allele frequency to be higher for the Holstein breeds that they examined, [3, 5-7]. Also, similar results were seen in previous studied other breeds (Table 1).

Genotype frequencies and allelic frequencies of Holstein cattle population are presented in Table 2 and Figure 1, respectively.

Table-2. The allelic and genotypic frequencies of the bGH/AluI and CSN3/HinfI gene polymorphism, heterozygosity and fixation index and the statistical test result for Hardy-Weinberg equilibrium.

	Genotype			Allele Frequency		Heterozygosity and Fixation index			H-W χ^2 test
	LL	LV	VV	L	V	Ho	He	F _{is}	
bGH	0.50 (93)	0.48 (89)	0.02 (4)	0.74	0.26	0.478	0.385	-0.24	10,818**
CSN3	0.71 (175)	0.23 (58)	0.06 (15)	0.82	0.18	0.234	0.294	0.20	9.797**

** P<0.01

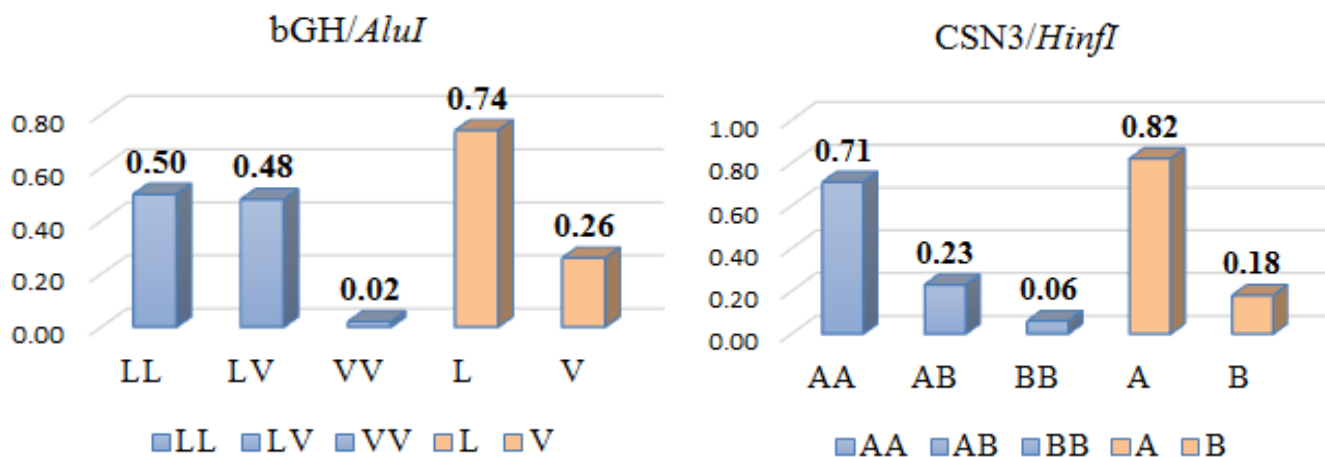


Figure-1. In Holstein cattle, bGH/AluI and CSN3/HinfI Polymorphism and allelic and genotypic states.

248 Holstein cattle in total were genotyped in the CSN3/HinfI region, and genotype frequencies were identified as 0.71 for AA, 0.23 for AB, 0.06 for BB, and allele gene frequencies for A and B alleles were calculated as

0.82 and 0.18 respectively. In many studies previously conducted on Holstein breed A allele frequency was observed to be higher [3, 7-11] and these results were similar to the results of present study (Table 1).

In studied Holstein population, heterozygosities were found in high rate as 0.478 in bGH region and in medium rate as 0.234 in CSN3/*HinfI* region and calculated F_{IS} values were -0.24 and 0.20 respectively, and both in the region, the population was not in Hardy Weinberg equilibrium (Table 2). These findings indicate that the population have been exposure to reproductive management and genetic improvement programs. Also, this case may have resulted from sampling error or animal transfers made into the herd from inside or outside.

4. Conclusion

As a result, PCR-RFLP, which is an economical, safe and fast method used for the detection of genetic markers, was successfully used in the determination of the 3 genotypes (AA, AB, and BB) of kappa casein and growth hormone (LL, LV, and VV) of organically raised Holstein cattle. But ultimately, no there are differences in gene frequencies with previously reported literature.

With the determination of some genetic markers, the indirect use of which in today's animal breeding is being discussed, the first step to achieve the desired efficiency target has been taken. The importance of this and other similar markers will increase only if they are used in relation to the correctly obtained efficiency features and with the aim of selection. With this purpose, there is a need in animal farming for the populations, the efficiency record of which is regularly kept, and the detection of genetic markers with high importance level is of high priority.

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