Allelic Frequency of Kappa-Casein, Growth Hormone and Prolactin Gene in Holstein, Brown Swiss and Simmental Cattle Breeds in Turkey^[1]

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Summary

The purpose of this study was to examine the kappa-casein (κ -CN), growth hormone (bGH) and prolactin hormone (PRL) gene polymorphisms in the Holstein (n=150), Simmental (n=50) and Brown Swiss (n=50) cattle breeds in Turkey. In order to determine the κ -CN-*Hind*III, PRL-*Rsa*I and bGH-*Alu*I polymorphisms, polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) were performed. A 443 bp fragment of κ -CN, a 223 bp fragment of bGH and a 156 bp fragment of PRL were amplified. In this study, two types of alleles, A and B for κ -CN, V and L for bGH, and A and B for PRL, were observed. The cattle breeds in which the highest frequencies of the alleles were estimated, were the HL breed (0.82) for κ -CN-A, the BS breed (0.55) for κ -CN-B, the HL breed (0.85) for bGH-L, the S breed (0.34) for bGH-V, the HL breed (0.87) for PRL-A, and the BS breed (0.24) for PRL-B. According to the results of the chi-square test, a significant deviation from the Hardy-Weinberg equilibrium was determined only for the bGH locus in the investigated breeds. The present study is the first report that examines three loci (κ -CN, bGH and PRL) in three cattle breeds of European origin (Holstein, Simmental and Brown Swiss) raised in Turkey.

Keywords: Cattle, Genetic polymorphism, Growth hormone, Kappa-casein, Prolactin

Türkiye'deki Holştayn, İsviçre Esmeri ve Simmental Sığır Irklarında Kapa-Kazein, Büyüme Hormonu ve Prolaktin Genlerinin Allel Frekansları

Özet

Bu çalışmada Türkiye'de yetiştirilen Holştayn (n=150), Simental (n=50) ve İsviçre Esmeri (n=50) sığır ırklarında kapa-kazein (κ-CN), büyüme hormonu (bGH) ve prolaktin hormonu (PRL) gen polimorfizimlerinin belirlenmesi amaçlanmıştır. Kapa-kazein-*Hind*III, PRL-*Rsa*I ve bGH-*Alu*l polimorfizimleri polimeraz zincir reaksiyonu ve restriksiyon parçacık büyüklük polimorfizimi (PCR-RFLP) ile belirlenmiştir. Kapa-kazein geni için 443 bç'lik bir bant, bGH geni için 223 bç'lik bir bant ve PRL geni için 156 bç'lik bir bant PZR ile yükseltgenmiştir. Bu çalışmada κ-CN için A ve B; bGH için L ve V; PRL için A ve B olarak olarak adlandırılan iki allel belirlenmiştir. En yüksek κ-CN-A allel frekansı HL ırkında (0.82), en yüksek κ-CN-B allel frekansı ise BS ırkında (0.55) belirlenmiştir. En yüksek bGH-L allel frekansı HL ırkında (0.85), en yüksek bGH-V allel frekansı ise S ırkında (0.34) belirlenmiştir. En yüksek PRL-A allel frekansı HL ırkında (0.87), en yüksek PRL-B allel frekansı ise BS ırkında (0.24) belirlenmiştir. Ki-kare sonuçlarına göre, incelenen ırklarda Harrdy-Weinberd dengesinden istatiksel olarak sapma sadece bGH lokusunda gözlenmiştir. Bu çalışma Türkiye'de yetiştirilen Holştayn, Simmental ve İsviçre Esmeri sığır ırklarında κ-CN, bGH ve PRL genotiplendirilmesinin yapıldığı ilk çalışmadır.

Anahtar sözcükler: Büyüme hormonu, Genetik polimorfizim, Kapa-kazein, Prolaktin, Sığır

INTRODUCTION

The accurate prediction of the future yields of breeder animals is one of the most significant, yet, complicated

issues of farm animal breeding. The lengthiness of the generation interval prevents the achievement of a rapid

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genetic improvement through the use of available selection methods in farm animal breeding. Therefore, researchers are striving to develop efficient methods for livestock, including cattle, sheep, goats and horses, which would aid in the selection of potential breeders within a shorter time period and with greater accuracy. In recent years, research on molecular markers and the correlation between gene polymorphisms and different yield traits have gained increasing importance in farm animal breeding ¹.

On the global scene, selection related research conducted in dairy cattle, generally focuses on milk yields, milk components and dairy technology². Milk yield is a polygenic trait, which is affected by environmental factors. In recent years, the presence of several genes, which determine the correlation between milk protein polymorphism and the physiological and biochemical traits affecting milk yield, has been reported. It has been indicated that the allelic structure of the genes coding several hormones, such as the growth hormone (GH) and prolactin (PRL), as well as milk proteins, such as kappa-casein (κ-CN), are correlated with milk yield, milk components and milk processing products. For this reason, it is considered that the allelic structure of the κ-CN, GH and PRL genes could be used as a candidate gene in predicting the lactation performance of potential bovine breeders².

In cattle, it has been ascertained that the κ -CN gene has 12 alleles, named as A, B, B2, C, E, F, F1, G, H, I, A(1) and J³. While the majority of these alleles have been determined to exist in only a few cattle breeds at a low frequency, it has been ascertained that the A and B alleles are very common and are found in almost all cattle breeds ³. It has been reported that, of the casein proteins, κ -CN has affect on milk yield, milk protein composition and milk fat content ⁴.

In addition to milk protein polymorphisms, another subject studied extensively in farm animals is the growth hormone gene ⁵. The growth hormone is involved in multiple physiological processes, including the regulation of growth, development of the mammary glands, onset of lactation, glucogenesis, activation of lypolysis, and regulation of muscle development ⁶. In view of the abovementioned effects of the growth hormone, both GH concentration and the allelic variations of the gene coding this hormone have drawn the attention of researchers. It has been reported that, in cattle, the bGH gene is correlated with certain yield traits, in particular with milk yield and quality, growth ⁶, carcass composition and quality ⁷. In this context, it is considered that it could be of use as a candidate gene in animal improvement programmes targeted at increasing milk and meat yields⁸. Previous molecular genetic studies have demonstrated that yield traits such as milk yield and body weight gain are correlated with certain polymorphisms of the bGH gene⁶.

In mammals, prolactin is responsible for the onset and maintenance of lactation, the growth of the mammary

glands, and lactogenesis ⁸. In view of the effects described above, it is considered that this gene could be used as a potential genetic marker of milk yield in cattle ⁸. However, information available on polymorphisms of the PRL gene in cattle remains limited. A few polymorphisms have been reported for the bovine PRL gene ⁹. Literature reports are available, which report correlation to both exist ⁸ and not exist ¹⁰ between PRL-*Rsa*l polymorphisms and several traits, including milk yield, milk fat rate and milk protein content in the particular animal species and breeds investigated. For, it is considered that the variants of this gene could be used in the development of an appropriate test method for genetic improvement programmes and animal breeding ¹⁰.

The present study was aimed at the determination of the allelic structure of the κ -CN, bGH and PRL genes for the first time in Turkey, using the restriction fragment length polymorphism (RFLP) method in the Holstein, Simmental and Brown Swiss breeds.

MATERIAL and METHODS

Cattle of the Holstein (HL, n=150, from Kayseri, Kahramanmaraş, Ankara), Simmental (S, n=50, from Kayseri) and Brown Swiss (BS, n=50, from Kayseri, Çorum) breeds, including those obtained by the Cattle Breeders Association of Turkey from several different farms in 2010, constituted the material of the study. The DNA used in the study was isolated using the phenol-chloroform extraction method. The determination of the κ -CN-*Hind*III, bGH-*Alu*I and PRL-*Rsa*I polymorphisms in the cattle breeds investigated was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described by Chrenek *et al.*¹¹.

The genotypic structure and allele frequency of the individual animals used in the trial were determined by means of gene counts. The Hardy-Weinberg equilibrium of the breeds included in the trial for the loci investigated was analysed using the Chi-square test. This statistical analysis was performed using the Pop Gene software package program version 1.32¹².

RESULTS

The PCR performed for the κ -CN gene produced a single band of 443 bp length for the samples assayed. Following enzymatic digestion with *Hind*III endonuclease for the kappa-casein gene, in animals of the homozygote BB genotype two bands of a length of 348 bp and 95 bp; in animals of the AB genotype three bands of 443, 348 and 95 bp length, and in animals of a homozygote AA genotype a single band of 443 bp length were observed (*Fig. 1*).

Following enzymatic digestion with *Hind*III for the kappacasein gene, the highest rates of the genotypes AA and



Fig 1. *Hind*III enzyme digestion products of different κ -CN genotypes. M 100 bp DNA ladder; 1, 3 and 6 AA (443 bp) individuals genotyped: 2 and 5 BB (348, 95 bp) individuals genotyped: 4, 7 and 8 AB (443, 348, 95 bp) individuals genotyped

Şekil 1. *Hind*III enzimi ile kesim sonucu elde edilen farklı κ-CN genotipleri. M 100 bç'lik DNA cetveli; 1, 3 ve 6 AA (443 bç) genotipli bireyler: 2 ve 5 BB (348, 95 bç) genotipli bireyler: 4, 7 ve 8 AB (443, 348, 95 bç) genotipli bireyler



Fig 2. *Alu*I enzyme digestion products of different bGH genotypes. M; 100 bp DNA ladder; 1 and 8; individuals of the LL (171, 52 bp) genotype: 4 and 5; individuals of the VV (223 bp) genotype: 2, 3, 6 and 7; individuals of the LV (223, 171, 52 bp) genotype

Şekil 2. Alul enzimi kesim sonucu elde edilen farklı κ -CN genotipleri. M 100 bç'lik DNA merdiveni; 1 ve 8; LL (171, 52 bç) genotipli bireyler: 4 and 5; VV (223 bç) genotipli bireyler: 2, 3, 6 and 7; LV (223, 171, 52 bç) genotipli bireyler

BB were determined in the HL and BS breeds, respectively, whilst the highest rate of the genotype AB was ascertained in the S and BS breeds. The analysis of the samples pertaining to the breeds investigated revealed that the frequency of the A allele was greater than that of the B allele in the S and HL breeds, whilst in the BS breed the frequency of the B allele was greater than that of the A allele. The genotype and allele frequencies of the cattle breeds investigated are shown in *Table 1*.

PCR performed for the growth hormone gene produced a single band of 223 bp length in the samples. Digestion of the PCR products obtained for the growth hormone gene with the enzyme *Alul* endonuclease resulted in a single band of 223 bp in animals of the VV genotype, three bands of 223, 171 and 52 bp length in animals of the LV genotype, and two bands of 171 and 52 bp length in animals of the LL genotype (*Fig. 2*).

According to the bands produced by the digestion of the PCR products pertaining to the samples analysed for the growth hormone gene by the enzyme *Alu*I endonuclease, the highest frequency of the genotype LL was determined in the BS breed, whilst the highest frequency of the genotypes VV and LV was determined in the S breed. In all of the cattle breeds investigated, the frequency of the L allele was higher than that of the V allele. However, the frequency of the V allele was highest in the S breed, compared to the other two cattle breeds investigated. The genotypes and alleles of the cattle breeds investigated in the present study are given in *Table 2*.

PCR analysis for the prolactin gene produced a single band of 156 bp length in the samples assayed. Digestion for the prolactin gene by *Rsa*l endonuclease resulted in a

| Breed | n | Genotype | | | | | | | Allele Frequency | | |
|-------|-----|----------------|------|--------------|------|--------------|------|--------|------------------|----------|-----------------------|
| | | AA | | BB | | AB | | | | χ2 | χ2 p-value |
| | | Obs (Exp) | F. | Obs (Exp) | F. | Obs (Exp) | F. | A | В | | L yaras |
| S | 50 | 27 (25.8182) | 0.54 | 5 (3.8182) | 0.10 | 18 (20.3636) | 0.36 | 0.7200 | 0.2800 | 0.694249 | 0.404723 ^N |
| BS | 50 | 9 (9.5556) | 0.18 | 15 (15.5556) | 0.30 | 26 (24.8889) | 0.52 | 0.4400 | 0.5600 | 0.101744 | 0.749746 ^N |
| HL | 150 | 105 (101.6087) | 0.70 | 8 (4.6087) | 0.05 | 37 (43.7826) | 0.25 | 0.8233 | 0.1767 | 3.659409 | 0.055753 ^N |

Table 2. Allele and genotype frequencies of the bGH locus in the S, BS and HL cattle breeds

| Breed | n | Genotype | | | | | | | Allele Frequency | | |
|-------|-----|----------------|------|------------|------|--------------|------|--------|------------------|--------|---------------|
| | | LL VV | | | LV | | | | V | χ2 | χ2 P-value |
| | | Obs (Exp) | F. | Obs (Exp) | F. | Obs (Exp) | F. | L | v | | |
| S | 50 | 25 (21.6667) | 0.50 | 9 (5.6667) | 0.18 | 16 (22.6667) | 0.32 | 0.6600 | 0.3400 | 4.434 | 0.035* |
| BS | 50 | 38 (35.2121) | 0.76 | 4 (1.2121) | 0.08 | 8 (13.5758) | 0.16 | 0.8400 | 0.1600 | 8.922 | 0.002** |
| HL | 150 | 115 (109.1639) | 0.77 | 9 (3.1639) | 0.06 | 26 (37.6722) | 0.17 | 0.8533 | 0.1467 | 14.693 | 0.000*** |



Fig 3. *Rsa*l endonuclease enzyme digestion products of different PRL genotypes. M; 100 bp DNA ladder: 1, 4 and 9; individuals of the AA (156 bp) genotype: 2, 3 and 8 individuals of the BB; (82, 74 bp) genotype: 5, 6 and 7; individuals of the AB (156, 82, 74 bp) genotype

Şekil 3. *Rsa*l enzimi ile kesim sonucu elde edilen farklı PRL genotipleri. M 100 bç'lik DNA cetveli; 1, 4 ve 9; AA (156 bç) genotipli bireyler: 2, 3 ve 8 BB; (82, 74 bç genotipli bireyler: 5, 6 ve 7; AB (156, 82, 74 bç) genotipli bireyler

breeds raised in Turkey, namely, the HL, S and BS breeds, for the κ -CN, PRL and bGH genes.

Kappa-casein (к-CN)

It has been reported that the alleles of the kappa-casein gene, excluding the A and B alleles, are found in only certain cattle breeds and at a rather low frequency ³. The most frequently observed alleles in cattle breeds, namely, the A and B alleles, have emerged consequential to a base change in the κ -CN gene ¹⁴. In domestic cattle breeds, the frequency of the κ -CN-A allele has been found to be highest in the HL, compared to the dairy breeds Jersey and Guernsey ¹⁰. Similarly, reports on HL cattle raised in the United Kingdom ¹⁵, Argentina ¹⁰ and Italy ¹⁶ point out to the frequency of the κ -CN-A allele being greater than that of the B allele. In agreement with these reports, it was ascertained in the present study that the frequency of the κ -CN-A allele

| | n | Genotype | | | | | | | Allele Frequency | | |
|-------|-----|----------------|------|------------|------|--------------|------|--------|------------------|----------|------------------------|
| Breed | | AA | | BB | | AB | | _ | | χ2 | χ2 P-value |
| | | Obs (Exp) | F. | Obs (Exp) | F. | Obs (Exp) | F. | A | В | | |
| S | 50 | 32 (32.7273) | 0.64 | 1 (1.7273) | 0.02 | 17 (15.5455) | 0.34 | 0.8100 | 0.1900 | 0.458480 | 0.498335 ^{NS} |
| BS | 50 | 27 (28.7879) | 0.54 | 1 (2.7879) | 0.02 | 22 (18.4242) | 0.44 | 0.7600 | 0.2400 | 1.951590 | 0.162416 ^{NS} |
| HL | 150 | 115 (114.3512) | 0.77 | 3 (2.3512) | 0.02 | 32 (33.2977) | 0.21 | 0.8733 | 0.1267 | 0.233304 | 0.629085 NS |

single band of 156 bp length in animals of the genotype AA, three bands of 156, 82 and 74 bp length in animals of the genotype AB, and two bands of 82 and 74 bp length in animals of the genotype BB (*Fig. 3*).

In the samples analysed for the prolactin gene, the highest frequency of the genotype AA was determined in the HL breed. In all three cattle breeds investigated in the present study, the frequency of the genotype BB was found to be equal. The highest frequency of the genotype AB was determined in the BS breed. While the frequency of the A allele was greater than that of the B allele in the HL breed, the frequency of the B allele was highest in the BS breed. The genotypes and the allele frequencies of the cattle breeds investigated in the present study are shown in *Table 3*.

DISCUSION

Today, in farm animal breeding, it is aimed to predict the genetic value of potential breeder animals with greater accuracy and to achieve rapid genetic improvement through selection. In this context, the hypothesis suggesting that variations in certain genes, which affect physiological processes, are correlated with variations in quantitative traits has made a breakthrough in animal improvement efforts ¹³. The present study was aimed at the investigation of the genetic structure of the three major imported cattle (0.82) was greater than that of the B allele in the HL breed. In a study conducted on milk protein polymorphism, Oner and Elmaci ¹⁷ reported that, in HL cattle raised in Bursa province, the frequency of the genotype AA was greater than that of the other genotypes. Similarly, in the present study, the frequency of the genotype AA was higher than that of the other genotypes. However, Oner and Elmaci¹⁷ reported that the animals they investigated displayed deviation from the Hardy-Weinberg (HW) equilibrium for the κ-CN locus. The underlying reason may be the material of the study comprising only HL cattle raised in Bursa province. In the present study, in which 150 cattle raised in Kayseri province and its vicinity was investigated, it was ascertained that the HW equilibrium was maintained for the κ-CN locus. However, no information was available on the phylogenetic relation between the individuals. In the present study, both female animals raised in the Kayseri province and female animals supplied by the Cattle Breeders' Association from different provinces were used. Particular attention was paid to avoid the animals being dam and daughter and progeny of the same sire. Thereby, it is considered that an appropriate representation of the HL breed raised in Turkey was achieved in the present study. Compared to studies conducted in the HL breed, there are very few literature reports available on the κ-CN gene in the S and BS breeds. In very few studies conducted in the BS breed, it has been reported that, differently from

the HL, the frequency of the κ -CN-B allele was higher than that of the A allele ¹⁵. In compliance with these reports, in the present study, in which 50 BS cattle were investigated, the frequency of the B allele (0.55) was higher than that of the A allele. Previous research has shown that, in the S, which is a dual-purpose breed, the frequency of the κ-CN-A allele is higher than that of the B allele. However, it has been reported that, differently from the HL, in the S, the difference between the frequencies of the two alleles is not big ¹⁸. In the present study, the frequency of the κ -CN-A allele (0.62) was greater than that of the B allele. However, this frequency was found to be lower than that of the HL (0.82). The data obtained for the alleles of the κ -CN gene in all three cattle breeds investigated in the present study were in compliance with data previously reported from different parts of the world.

In research aimed at the genetic characterization of cattle breeds, it was determined that the frequency of the κ -CN-B allele was higher in breeds originating from the *Bos taurus*, compared to breeds originating from the *Bos indicus*¹⁹. These results show that data related to κ -CN gene polymorphism could be used in the genetic identification of cattle breeds as well as in the determination of the genetic origin of breeds and the genetic relations between different breeds. However, the frequency of neither the κ -CN allele nor genotypes should be assessed alone when determining differences or phylogenetic relations between breeds. For such targets, other loci should also be assessed. The present study is the first research, in which the κ -CN alleles have been determined in the HL, S and BS breeds raised in Turkey.

Growth Hormone (bGH)

In cattle breeding, it has been reported that polymorphisms of the bGH gene are correlated with milk yield traits such as milk yield and milk composition ²⁰ as well as with meat yield traits such as carcass composition and quality²¹. In the bGH gene, enzymatic digestion with Alul following PCR amplification has revealed the presence of two alleles, namely, the V and L alleles ¹, whilst enzymatic digestion with Mspl has revealed the presence of two alleles, namely, the +/- alleles 7. In the present study, which was aimed at the investigation of the allelic structure of the bGH gene in HL, S and BS cattle raised in Turkey, digestion was performed using the enzyme Alul. In the HL cattle raised in Poland ²⁰, Hungary ², Russia ¹⁰ and Australia ²², it has been determined that the frequency of the bGH-L allele is higher than that of the V allele. Similarly, in the present study, in which 150 female HL were investigated, the frequency of the bGH-L allele (0.85) was found to be higher than that of the V allele. In previously conducted studies, the frequency of the genotype LL has been reported to be higher than that of the other genotypes ^{2,20}. Similarly, in the present study, it was ascertained that the frequency of the genotype LL (0.77) was higher than that of the other genotypes. On the other hand, previous studies have shown that in the S and

BS breeds, the frequency of the bGH-L allele is higher than the frequency of the V allele ²³. Similarly, in the present study, the frequency of the L allele was higher than that of the V allele in both the S (0.66) and the BS (0.84) breeds. Of the three cattle breeds investigated, the S displayed the highest frequency for the genotype LV. In another study, in which of the milk-type cattle breeds, the HL, and of the meat-type cattle breeds, the Limousine, Charolaise, Piemontese, Angus and Hereford were investigated for the bGH locus, it was determined that the frequency of the L allele (0.86) was higher in the HL, whilst in the meat-type breeds the frequency of the V allele was higher (0.62)¹⁰. These results show that, in selection programmes, the bGH locus could be used to select breeder animals with a genotype appropriate for the type of breeding. It has been reported that, the S, which is of the LV genotype, is characterized by greater body weight gain and richer carcass composition, and has a V allele frequency of 0.32²⁴. Similarly, in the present study, in the S, the frequency of the V allele (0.34) was higher than that of the other two cattle breeds investigated.

Furthermore, in a study conducted in seven cattle breeds raised in Brazil and originating from the *Bos taurus* and *Bos indicus*, which were investigated for the bGH gene, in the cattle breeds originating from the *Bos taurus*, two alleles, namely, the L and V alleles were determined to exist; whilst the cattle breeds originating from the *Bos indicus* were monomorphic and possessed only the L allele ¹⁰. In another study, it was demonstrated that, in cattle breeds originating from the *Bos indicus*, the L allele was either monomorphic or had a higher frequency than that of the V allele ²⁵. Therefore, it is considered that the bGH locus could be used in research on the origin of breeds.

Prolactin (PRL)

The bovine PRL locus is reported to have two alleles, namely, the A and B alleles 8. Previous research has shown that in HL cattle raised in Russia, South Korea ¹⁵ and Lithuania²¹ the frequency of the PRL-A allele is higher than that of the B allele. In the HL cattle investigated in the present study, the frequency of the PRL-A allele (0.87) was higher than that of the B allele. In BS cattle raised in Slovakia, it was found out that the frequency of the PRL-A allele was higher than the frequency of the B allele, yet, the frequency of the A allele was not as high as that determined in cattle of the HL breed ⁴. In the present study, in the 50 BS cattle investigated, the frequency of the PRL-A allele (0.76) was found to be higher than that of the B allele, yet of the three cattle breeds investigated, the BS displayed the highest frequency for the PRL-B allele (0.24). Again, in the present study, in samples pertaining to the 50 S cattle investigated, the frequency of the PRL-A allele (0.81) was higher than the frequency of the B allele. The number of studies on the PRL gene in the S is rather limited. Therefore, it is considered that the results of the present study will contribute to literature by providing

data on the allelic structure and allele frequencies of the PRL gene in the S breed.

The association between bGH genotypes and milk yield traits has been reported in cattle ⁵. It is considered that the bGH-Alul polymorphism can be used as a potential marker for the milk yield traits ¹⁰. The polymorphism of the κ -CN gene has been studied extensively in cattle breeds ^{26, 27}. The relationship between the κ -CN alleles and some milk yield traits such as the total protein content of milk, fat percentage of milk and milk production, has been reported in previous studies ²⁸. The κ -CN-B allele has been indicated to have a significant effect on milk yield traits ¹⁶. Also, it has been reported that the κ -CN-B allele has a favourable effect on technological milk properties ²⁶. Several authors have examined the effect of the PRL-*Rsa*I AA, AB and BB genotypes on some milk yield traits ¹⁰. The highest milk and milk fat yields were obtained in cows of the BB genotype ¹⁹.

Currently available data is not sufficient to confirm the use of the κ -CN, PRL and bGH gene polymorphisms as markers for selection in cattle breeding. Further studies are required to determine the correlation between the variants of the genes coding the κ -CN protein and bGH and PRL hormones, which affect milk yield, and milk yield parameters. Furthermore, it is considered that these variants could be used for the development of appropriate test systems for use in genetic research and animal breeding programmes. The present study has demonstrated, for the first time, polymorphisms of the κ -CN, PRL and bGH genes in HL, S and BS cattle raised in Turkey.

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